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THE ANATOMY OF *SIPHONOPHORA ROSARUM*, WALK., THE "GREEN-FLY" PEST OF THE ROSE-TREE.

PART I. THE APTEROUS VIVIPAROUS STAGE.

BY A. J. GROVE, M.Sc., *Research Scholar, Zoological Laboratory, University of Birmingham.*

(Plate I and 15 Text Figures.)

CONTENTS.

Introduction.

WHEN beginning this investigation it was my intention to make a comparison between the anatomy of the apterous viviparous form and that of the winged viviparous form, to ascertain what differences were present corresponding with the difference in habit of the Aphis in these two stages. I found, however, that the literature bearing on the group to which *S. rosarum* belongs, was somewhat scanty, and as a rule, rather out of date. It therefore became necessary, before undertaking the

comparative work, to obtain a somewhat more complete account of the general anatomy. It is with this idea in view that the following paper has been written; but, as will be seen on perusing it, much more attention has been given to the internal anatomy than to the external parts. It is my intention, now that this first part of the task is completed, to take up my original project. I desire to take this opportunity of expressing my gratitude to Professor Bridge, Mr A. E. Shipley, F.R.S., Mr Doncaster and Mr Collinge for the valuable assistance and advice they have given me whilst this work has been in progress.

Methods.

The extremely small size and delicacy of the insects dealt with entailed somewhat careful manipulation. To obtain a good supply of material, rose trees were infested, as far as possible, with *S. rosarum* only. In this way a fresh supply of good material was always ready at hand. For fixing, Petrunkewitsch's modification of Gilson's fluid was used, the formula being:

Water (distilled)	30 parts.
Absolute alcohol	20 parts.
Glacial Acetic Acid	9 parts.
Nitric Acid (pure)	1 part.

Corrosive sublimate to saturation.

For staining the sections, Naples Carmine, Ehrlich's Haematoxylin, and Weigert's Elastic tissue stains were used.

In order to confirm results obtained from the serial sections dissections were made; and for this purpose I found the large binocular dissecting microscope made by Zeiss of the utmost use. The microscope was the one having the alterations suggested by Braus and Drüner, and the large working distance possessed by its lenses gave great facility in work performed under it. The instrument is to be strongly recommended for such minute work as the dissection of Aphides. The dissections were carried out in glycerine.

Whilst engaged on the tracheal system, I found that a very effective way of showing up the details of the system was to kill and mount a few specimens in hot glycerine jelly. The effect was that the jelly in cooling, stopped up the stigmata, preventing the escape of the air, and yet penetrating sufficiently to render the animals transparent. Care must be taken not to overheat the jelly, or the heat will drive the

air from the tracheae before the jelly has solidified sufficiently to stop up the stigmata.

Whenever possible, the drawings were made with the camera lucida, and in the diagrammatic ones the proportions were obtained from camera lucida sketches.

External characters.

Buckton (1875—1882), in his Monograph, gives the distinguishing features of *S. rosarum* as follows:

Long, oval, flat. Wholly green, ringed, the posterior edges fringed with capitate hairs which mostly spring from small tubercles. Vertex tufted. Antennae and legs somewhat short. Frontal tubercles small. Eyes reddish brown. Tail green, and not conspicuous.

This description certainly includes most of the distinguishing features of *S. rosarum*; and it is only intended here to give in more detail points not brought out in the above description.

The body consists of head, thorax and abdomen; the first two together forming the anterior half of the body and the abdomen the posterior half (Text Figs. 1 and 2).

The segmentation of the body is not so markedly clear as Buckton seems to infer. The three segments of the thorax are fairly distinct, and, on the ventral surface only, the segments of the abdomen can be made out, but they are almost invisible on the dorsal surface.

When the Aphis is viewed from above, the most striking feature is the rows of capitate hairs (*cap. h.*) which Buckton mentions. These are arranged in six rows—three on each side of the median line (Text Fig. 1). The two middle rows are more prominent than the others from the fact that the tubercles or excrescences upon which the hairs are mounted, are more pronounced than in the other cases. Moreover, in these median rows each tubercle bears two hairs and the rows are continued right to the front of the head. In the other cases the rows are confined mainly to the abdomen. These hairs are also found for a short distance on the legs, usually ceasing at the beginning of the tibia, so that towards the end of that joint the hairs are of the usual tapering kind. A remarkable feature about the feet of the Aphis is the reduction of the number of the tarsi to one (Text Fig. 4). This is described by Buckton. The antenna (Text Fig. 5) of *S. rosarum*—in common with all the genus *Siphonophora*—consists of six joints. The last joint is very peculiar, it being composed of two parts—an expanded

portion forming the base and terminating in a more slender part. The whole of the surface of this joint is covered with small ridges giving it quite a remarkable appearance (Text Fig. 6). The significance of these ridges is open to conjecture, but probably they indicate the presence of some kind of sense organ.

The only definite sense organs which *S. rosarum* has are the eyes,

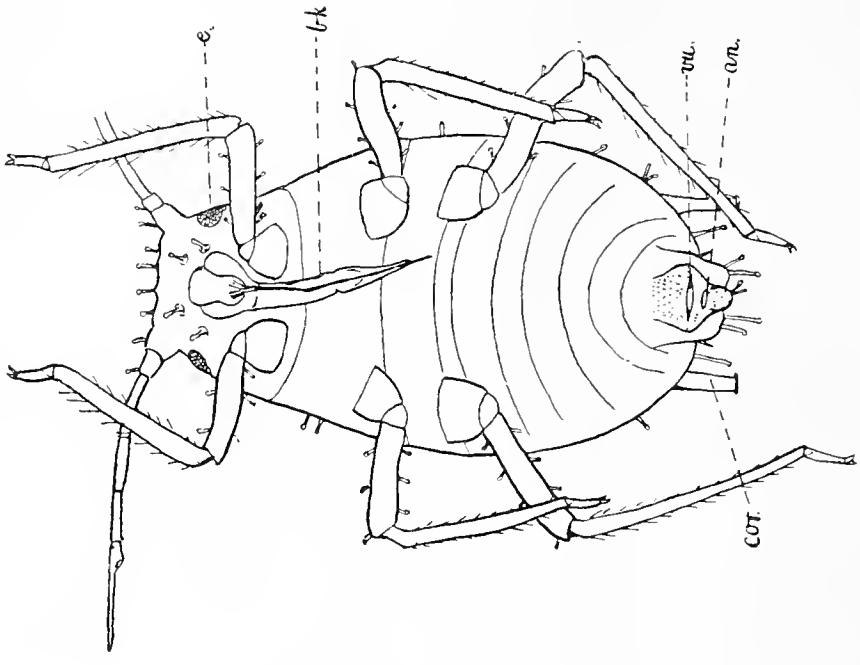

Fig. 2. *Siphonophora rosarum.*
Ventral view. (×26.)
an. = anus. *bk.* = beak or proboscis.
cor. = cornicle. *e.* = eye. *vu.* = vulva.

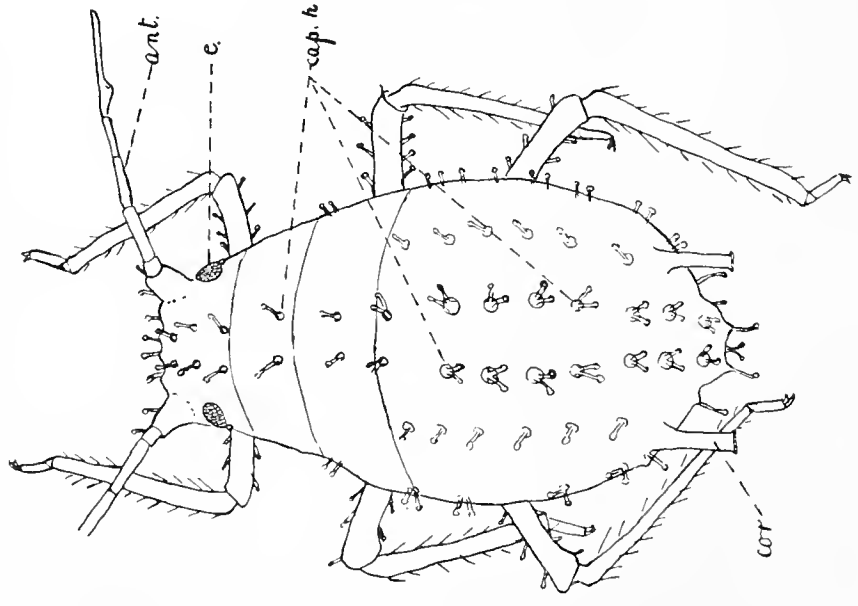

Fig. 1. *Siphonophora rosarum.*
Dorsal view. (×26.)
ant. = antenna. *cap. h.* = capitate hairs.
cor. = cornicle. *e.* = eye.

and these present no differences from those of other Aphides. Behind each eye is a small ocellus, or supplementary eye, possessing usually two facets (Text Fig. 7). Each eye and ocellus is supplied by a large nerve (*n. e.* Plate I, Fig. 20) from the optic lobe of its side. This nerve expands at the base of each eye, and from this expansion, nerve fibrils are given off to the ommatidia of the eye and ocellus.

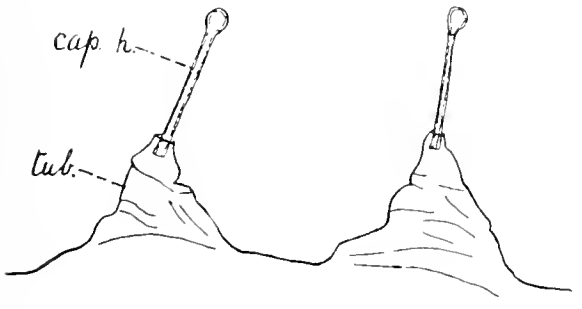

Fig. 3.

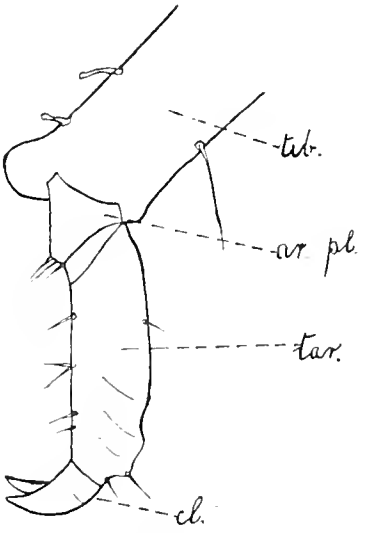

Fig. 4.

Fig. 3. Two capitate hairs and the tubercles from which they spring seen in profile. (×230.) *cap. h.*=capitate hair. *tub.*=tubercle.

Fig. 4. Tarsus of third leg. (×355.) *ar. pl.*=articulatory plate of the tarsus. *cl.*=claw. *tar.*=tarsus. *tib.*=tibia.

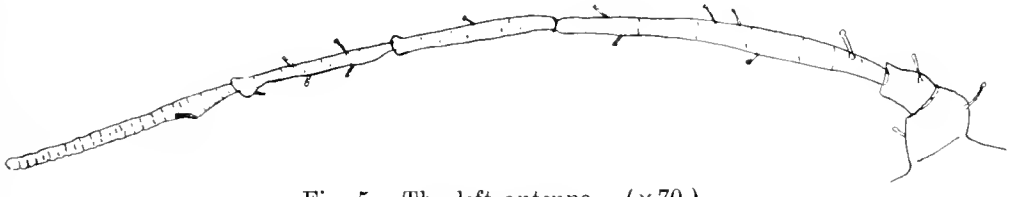

Fig. 5. The left antenna. (×70.)

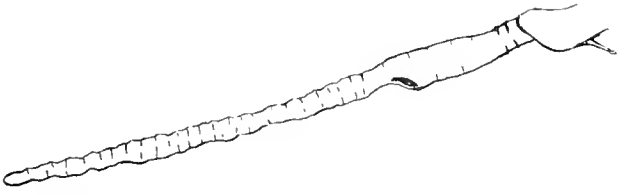

Fig. 6. The last joint of the antenna to show its remarkable appearance. (×152.)

The tail or cauda is, as Buckton says, quite inconspicuous, and is scarcely visible from the dorsal surface. It is completely covered with short stumpy hairs, which also surround the anal and reproductive apertures.

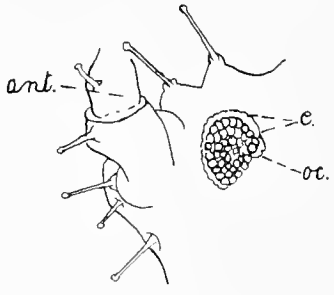

Fig. 7.

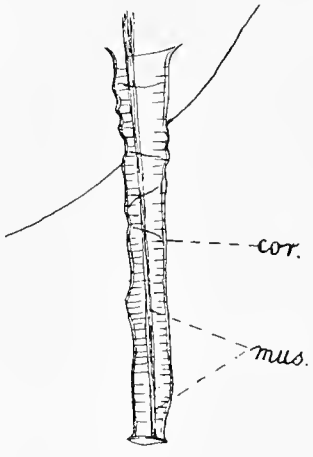

Fig. 8.

Fig. 7. Lateral view of eye and ocellus. (×108.)
ant. = antenna. *e.* = eye. *oc.* = ocellus.
Fig. 8. A cornicle or nectary. (×100.)
cor. = cornicle. *mus.* = controlling muscle.

The Alimentary Canal.

The alimentary canal of Aphides in general has been figured from time to time by various authors, among whom may be mentioned Dufour (1833), Buckton (1875—82), Witlaczil (1882), and others.

Witlaczil's figure of the alimentary canal of *Aphis pelargonii* agrees in many points with the results I have obtained.

The alimentary canal presents many remarkable features, the most striking of which is the complete absence of pyloric caeca or malpighian tubules. Dufour, among the earliest investigators of this part of the anatomy, expresses his astonishment thus:—

"L'appareil digestif des Pucerons présente une exception fort remarquable, unique.....; c'est l'absence absolue des vaisseaux hepatiques."

The alimentary canal (Text Figs. 9 and 10) commences at the oral aperture which is situated on the lower posterior side of the head, and is so placed that it lies in close connection with the groove which traverses the whole length of the upper surface of the proboscis or beak,

and which receives the setae after they leave the head. The mouth opens into an extremely narrow oesophagus which extends first in a vertical direction towards the dorsal surface of the head, passing on its way through the loop made by the circum-oesophageal connectives. On

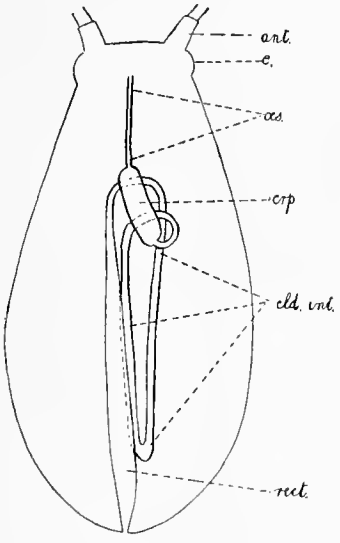

Fig. 9.

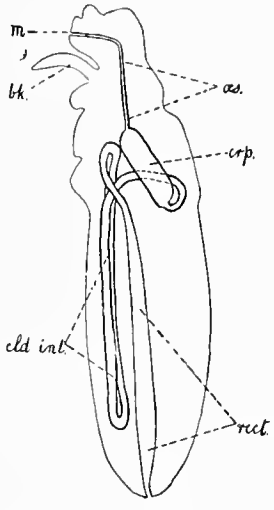

Fig. 10.

Fig. 9. Dorsal view of alimentary canal. Semi-diagrammatic. (×20.)
ant. = antenna. *cld. int.* = coiled intestine. *crp.* = crop. *e.* = eye. *rect.* = rectum.
Fig. 10. A lateral view of the alimentary canal. Semi-diagrammatic. (×20.)
bk. = beak or proboscis. *cld. int.* = coiled intestine. *crp.* = crop.
m. = mouth. *oes.* = oesophagus. *rect.* = rectum.

reaching a position about two-thirds of the distance from the ventral to the dorsal surface, the oesophagus turns abruptly backwards and continues in the median line to a position in the anterior portion of the mesothorax. At this point—in an external view—the narrow oesophagus dilates abruptly to form the crop or stomach, but, seen in section, the oesophagus is continued for a short distance into the interior of the crop, thus causing a slight invagination of the anterior end of the latter. In this way an effective valve is formed to prevent the fluid poured into the crop passing back again into the oesophagus. This point was observed and figured by Witlaczil (1882), and is shown in Fig. 13 (Plate I). The crop is the widest portion of the alimentary canal, and generally lies in an oblique position, its posterior end pointing towards the right and dorsal sides of the body. The crop then gives place to the coiled intestine which is considerably narrower than the crop, but has a lumen very much larger than that of the oesophagus. The curves

of this part of the alimentary canal vary in different individuals, and in many cases they are complicated by the contraction of the abdominal segments at death. In the Text Figs. 9 and 10 are represented diagrammatically coils which are present in every individual, but complications in the form of smaller supplementary coils may occur. Commencing at the crop, the coiled intestine inclines towards the right, and in doing so, curves completely round, dips down, passes beneath the crop, and reaches a position on the ventral surface, slightly to the left of the median line. From this point, it proceeds in a posterior direction to about the middle of the abdomen; then, curving abruptly, returns forwards until the region of the crop is again reached. Here it makes its final curve, and rising above the last-mentioned coil, takes a direct course to the anus which is placed at the posterior end of the body. In this last coil the intestine merges into the rectum, which at first widens gradually, beginning to narrow again as it approaches the anus. The supplementary coils mentioned above usually occur where the alimentary canal makes its backward curves.

The *mouth* is bounded by four separate structures each contributing towards the formation of the oral cavity. The anterior margin is bounded by the pointed labrum (see Plate I, Fig. 12), and the posterior margin is formed by a part of the labium which takes the form of a small under-lip, while the remainder of the labium constitutes the beak or proboscis. Laterally, the mouth is bounded by two down-growths from the sides of the head, and these fit in between the labrum and the small under-lip,—the whole enclosing the small oral cavity. The setae in their course out of the head pass through this oral cavity, and the labrum has a marked groove on its inner side in which they are lodged before they leave the head altogether to lie in the groove in the upper surface of the beak.

The *mouth organs* of *S. rosarum* agree in some points with those of other—and in some cases more specialised—Aphides, described by Witlaczil (1882), Krassilstschik (1892 and 1893), Drefus (1894), etc. One point of difference, however, is that my investigations fail to reveal any definite organ which is at all comparable to the salivary pump described and figured by them in other genera.

Witlaczil (1882), in his description of the mouth organs of Aphides, leaves several points somewhat obscure, more especially with regard to the number and condition of the setae. The setae of all the Hemiptera have attracted a great deal of attention, because there seems to be some doubt as to their true homology; but it is not intended in this paper to

enter into that discussion, but rather to give as accurate an account as possible of the mouth organs of the species under consideration.

S. rosarum, like other Aphides, possesses a chitinous framework in the head for the support and control of the mouth organs. Whether this corresponds to the tentorium usually present in the head of insects is rather doubtful; at least it is much more complex than the usual type of tentorium. Fig. 1 (Plate I) gives a general frontal view of this chitinous framework, and it agrees in some points with the figures given by Witlaczil and Krassilstschik; but, at the same time, there are points of difference, especially in connection with the lower parts.

It will be necessary first to describe in detail this framework, and in doing so, reference will be made to similar arrangements in other Aphides.

The chitinous framework. Traversing the head from side to side, in a position about two-thirds of the distance, dorso-ventrally, from the ventral side of the head, there is a strong, hollow, slightly curved rod of chitin, which is closely applied to the under surface of the cerebral ganglia (see Plate I, Fig. 4). From this rod the whole of the chitinous framework depends, and to it most of the muscles controlling the mouth parts are attached. Compared with the figures of Mark (1877), Krassilstschik (1892 and 1893), etc., this rod corresponds with their "Arcus superior," but I think the term "Transverse bar" is more appropriate. This bar has two pairs of chitinous supports, which may be termed the "Anterior and Posterior lateral rods," one pair arising from its anterior face, and the other pair from its posterior face. These supports arise from the ends of the bar, and consist of slender hollow rods of chitin, joining the bar to various parts of the head. The anterior lateral rods pass almost horizontally forwards, closely applied to the under surface of the expanded portion of the cerebral ganglia, so that the tapering portions of the ganglia lie between them. This pair eventually becomes continuous with the chitin of the front of the head. The posterior lateral rods dip down at a considerable angle and finally become connected with the ridge which marks the junction of the head with the first thoracic segment.

Depending from the centre of the tranverse bar or rod are two rod-like pieces of chitin which are arranged in the form of an inverted V (Λ), the junction of the two arms corresponding with their point of attachment to the transverse bar. The arms of the Λ-shaped bar are divided towards the lower end by a kind of joint, and finally they both make a strong curve inwards and become continuous with the chitin of

the floor of the front of the head. The lower part of each arm—that is from the joint downwards—has attached to it two curved rods of chitin, placed one above the other, and projecting one forwards and one backwards into the head. The more dorsally placed rod from each side is continuous with the expanded base of the anterior seta of that side, and the lower rod from each side is attached to the base of the posterior seta. In this way, the transverse bar, the arms of the Λ, and the setae themselves are all in direct continuity with one another. This point will be dealt with again in connection with the protrusion of the setae.

In the description of the pharynx it will be shown that the posterior wall of the pharynx is strengthened by a strong layer of chitin which extends from the mouth aperture to the point where the oesophagus makes its abrupt curve over the transverse bar, and in preparations to show the chitinous framework, this strip of chitin always remains behind and hence it appears in Fig. 1 (Plate I), though it does not actually contribute to the support of the mouth parts. The chitinous skeleton of the front of the head is raised into an ovoid dome, and on the inner surface of the dome the muscles which dilate the pharynx have their origin.

The setae. With regard to these organs, *S. rosarum* agrees, so far as I am able to judge from the literature at my disposal, with most of the Aphides. The setae are really four in number, but, as Buckton and others have observed, the proboscis or beak only contains three stylets. Witlaczil (1882), figures (Plate II, Fig. 5) *Aphis platanoides* with four setae in the beak, but I feel sure that this must be an error. Krassilstschik (1893) states that in *Phylloxera vestatrix*, fusion of two setae has taken place, but he does not make it quite clear which pair of setae have fused.

In *S. rosarum* the setae are arranged in two pairs—an anterior and a posterior pair—and it is the posterior pair which fuse to form a single median seta-like structure. In Fig. 2 (Plate I) will be seen a transverse section across the upper lip or labrum, just where the setae are leaving the head to pass into the groove in the dorsal side of the beak. The three setae are all cut across transversely, and lie in a straight transverse line. On closer examination it will be seen that each of the two external setae has a single clear spot in the centre, which represents a fine tube traversing the whole length of the seta. On looking at the section of the median seta, it will be noticed that there are two clear spots, showing that there are two tubes running down it. This in itself is very good evidence that the median structure

represents two fused setae. Beyond this, however, the examination of sections transverse to the whole animal, shows that the two posterior setae approach one another and fuse in the middle line, at the same time coming forward, so that the product of their fusion lies between and separates the two anterior setae.

The muscles of the setae. Inserted in the base of each seta is a strong muscle which has its origin at the end of the transverse bar which crosses the head (Plate I, Fig. 4). The muscles, from their position, are obviously retractor muscles, their function being to assist in the withdrawal of the setae from the host plant. These muscles were figured accurately by Witlaczil (1882), but Krassilstschik (1893) states that the retractor muscles in *Phylloxera* are attached to the front of the head. These authors also figured protractor muscles, and Krassilstschik states that they are attached to the chitinous rods which project from the lower part of the arms of the Λ. In *S. rosarum* I have been unable to find any protractor muscles except a small one attached to each of the posterior setae, but these are quite short and wholly inadequate to perform the function of driving the setae into the host plant, and moreover, these muscles are much smaller than the retractor muscles. Surely it is anomalous to have the greatest force to do the least work, for it is obvious that more force is required to drive the setae into the tissues of the host plant than to withdraw them.

How then are the setae protruded?

The following is a suggestion as to the way in which the operation is performed, but first it will be necessary to give an account of the relations of the muscles in the head to the chitinous framework and the setae.

It will be remembered that in the introduction to the section on mouth parts, it was intimated that *S. rosarum* does not possess a salivary pump such as has been described by Mayer (1874 and 1875), Krassilstschik (1892 and 1893), Drefus (1894), and others. There are structures, however, which are identical in position and general appearance with those described by the above authors, but it seems to me that they serve an entirely different purpose.

In exactly the same position in the small pointed under-lip (probably part of the labium) which closes the mouth on its posterior margin, where the above authors have described the salivary pump, is a small U-shaped rod of *solid* chitin (Plate I, Fig. 7). Moreover, this structure is continuous with the chitin of this part of the head, so

that it is practically immovable. Now stretching between this U-shaped rod of chitin and the transverse bar which crosses the head, there is a very large double muscle. Where this muscle becomes continuous with the transverse bar, it splits up into a number of fine tendon-like slips (Plate I, Fig. 6), just in the same way as the pharyngeal muscles do at their insertion into the anterior pharyngeal wall. From this it may be inferred that the muscle takes its origin from the U-shaped rod of chitin, and that it is inserted into the transverse bar.

This being the case, what will happen when this muscle contracts? One of two things must take place. Either the small under-lip will be drawn up, or the transverse bar will be pulled down. The former is scarcely likely to happen, because the muscle does not lie in the right direction to produce this effect; but more than this, the origin of the muscle is in this under-lip. The only other alternative then is that the transverse bar will be pulled down. The idea that it is the transverse bar which is moved, is supported by the fact that, situated on either side of the middle point of the bar, is a muscle stretching between the bar and the roof of the head (Plate I, Fig. 5), and the only function which these muscles can have, is to restore the bar to its original position after some displacement. It is true that these muscles are small compared with the displacing muscle, but, as will be seen later, they are probably quite strong enough to perform the work they have to do.

Having now described the arrangement and function of the various muscles, we will return to the original problem of how the setae are controlled. First, the strong muscle inserted in the centre of the transverse bar contracts, pulling the bar downwards. The bar in its course will carry down with it the Λ-shaped rods attached to its centre (see Plate I, Fig. 1), and these in their turn will again bring pressure to bear upon the lower jointed parts (*j* in Plate I, Fig. 1) of the Λ. Now, as has been described before, the rods of chitin attached to this lower part are continuous with the bases of the setae, and my suggestion is that the upper parts pressing on the lower parts of the Λ-shaped rods cause, by the bending inwards of the lower parts, the chitinous rods to take up a lower position in the head, and at the same time carry the bases of the setae along with them; the total effect being, that the setae are protruded more or less vigorously, depending upon the force with which the muscle (*l. pr. m.*) contracts.

The fact that the posterior pair of setae have a small protractor muscle, does not interfere with the general working of the apparatus,

because on looking at Fig. 1 (Plate I) it will be seen that the chitinous rods attached to the bases of these setae are much weaker than those attached to the anterior setae, and it may be that some extra force is required to drive the fused setae into the host plant. From these considerations the name "Large Protractor Muscle" will not be inappropriate for the large muscle which is inserted into the transverse bar.

The next question for consideration is, how are the setae withdrawn? This is probably performed in the following way. First the large protractor muscle will relax. This will cause the chitinous rods, which were probably slightly bent during the process of driving in the setae, to regain their original position by their own elasticity, and then the two muscles attaching the transverse bar to the roof of the head will contract a little and raise the bar a trifle.

The next process is the loosening of the setae in the wound. This will of course be done by the retractor muscles attached to the bases of the setae (Plate I, Fig. 4). It is true that to do this, the retractor muscles will have to bend the chitinous rods attached to the bases of the setae; but it must be borne in mind that the transverse bar, where these muscles take their origin, is stronger than the chitinous rods, so that the muscles can bend the rods without disturbing the transverse bar. Another serious difficulty is whether the setae would be so tightly held in the host plant, that the effect of the contraction of the retractor muscles would be to drag down the transverse bar with the whole chitinous framework attached to it. It must be remembered, however, that the transverse bar is supported in the head by the two pairs of chitinous rods which proceed from either end and are attached to various parts of the head, and also that there are the two muscles stretched between the middle portion of the bar and the roof of the head. Surely these supports will hold the transverse bar rigidly enough for the loosening and partial withdrawal of the setae! Now that the setae are loosened, the only thing that remains to be accomplished is their complete withdrawal, and this will be effected by the two muscles attaching the transverse bar to the head contracting, and thus restoring the whole of the chitinous framework to its original position.

It is highly probable that when piercing the rose-tree, the Aphis does not drive in its setae as one might push an awl into a block of wood, but that the action consists of a continual driving in and subsequent withdrawal or partial withdrawal, for in this way the wound would be continually enlarged, and the movement of the setae in the wound would keep it open and so facilitate the flow of sap.

From the above account it will be seen that no very close comparison can be made between the mouth organs of *S. rosarum* and those of the other Aphides which have already been figured. The only conclusion one can come to is that there are wide differences between the various groups of Aphides in this respect. Of course one would expect to find some variations, especially in those Aphides which form galls, but it is remarkable that such wide differences should exist in such points as the presence or absence of a protractor muscle to each seta, and also the point of origin of the retractor muscles.

The *pharynx*—the first portion of the oesophagus—is modified, as in all the Hemiptera, to fulfil a suctorial function. It extends from the oral cavity to where the oesophagus turns backwards. The posterior wall of the pharynx is strengthened by a strong layer of chitin, while the anterior wall consists of a flexible but elastic membrane (see Plate I, Fig. 12). Originating from the front wall of the head, and inserted into the anterior wall of the pharynx, where they split up into a number of tendon-like slips, is a double series of muscles. These muscles, contracting, will pull the anterior wall outwards, in that way increasing the capacity of the pharynx, and the oesophageal valve preventing the return of fluid from the crop into the oesophagus, fluid, drawn up the groove in the beak by capillarity, will be sucked up through the mouth into the pharynx.

The muscles then relaxing, the membrane, by virtue of its own elasticity, will return to its former position; and this process commencing at the oral end of the pharynx, the excess of fluid will be gradually forced along the oesophagus into the crop. In this way the insect is able to suck up the sap of the plant which oozes from the wound made by the setae.

The *oesophagus* presents no remarkable features, structurally, beyond the fact that nuclei and cell boundaries are almost entirely absent—I do not find the series of nucleate cells figured by Witlaczil (1882). The wall is comparatively thick, and composed of fine granular material (Plate I, Fig. 8). The point for remark about the oesophagus, however, is its extremely small size, and, as a natural consequence, the almost ultra-capillarity of the lumen. Dufour (1833) was evidently struck by this fact, for he says in describing this portion of the alimentary canal: "L'œsophage a une ténuité plus que capillaire."

The *crop* consists of a thick-walled, cylindrical sac or bag for the reception of the fluid sucked up from the host-plant by the Aphis. The wall is composed of rather large cells with large nuclei and nuclear bodies, and somewhat granular cell-contents (Plate I, Fig. 9). The

interior of the crop appears to be always full of fluid which, in the prepared sections, appears as fine granular matter with large clear interspaces. This frequently gives a false impression of a solid crop. This point is illustrated in Fig. 13 (Plate I), which is a camera lucida drawing of a longitudinal section through such a crop.

The *coiled intestine* is the longest portion of the alimentary canal, and throughout its entire length it is roughly oval in cross-section, being flattened dorso-ventrally (Plate I, Fig. 10). The walls are comparatively thick, and large nuclei with definite nuclear bodies occur at frequent intervals. Cell boundaries, however, are not at all clear, and the nuclei are embedded in a matrix of rather coarse granular matter. This fact, coupled with the large size of the nuclei, suggests a secretory function for this portion of the alimentary canal; and in the complete absence of definite digestive glands, the probability is that this is so.

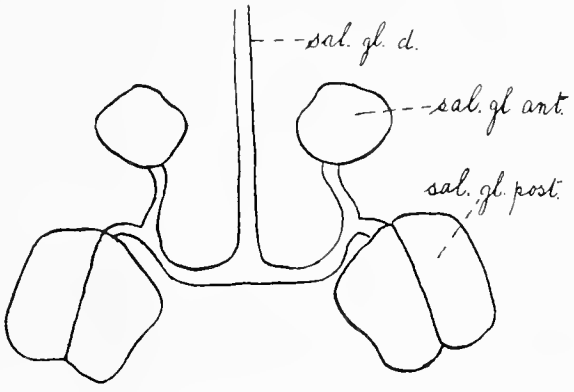

Fig. 11. Dorsal view of the salivary glands. Semi-diagrammatic. (×152.)
sal. gl. ant. = anterior salivary gland. *sal. gl. d.* = duct of salivary gland.
sal. gl. post. = posterior salivary gland.

The *rectum* differs greatly from the coiled intestine in structure. For the greater part of its length, it is composed of a network of longitudinal and circular bands of muscle fibres, the circular bands being on the outside (Plate I, Fig. 14). The wall is very much thinner than that of the coiled intestine, and there are only a few scattered nuclei present (Plate I, Figs. 11 and 14). Towards its posterior end, where the rectum narrows, the wall becomes much thicker and is thrown into folds, thus forming a sphincter muscle for the closing of the alimentary canal.

The *salivary glands* (Text Fig. 11) of *S. rosarum* are remarkably developed, considering the size of the insect. They consist of two pairs

of unequal size, the anterior pair being smaller than the posterior; and they lie—in close relations with the nervous system—partly in the hinder portion of the head, but mainly in the pro-thorax. Viewed from above, the larger posterior pair lie just in front of, and above, the first pair of thoracic ganglia; while the anterior pair take up a latero-dorsal position,—one on each side of the sub-oesophageal ganglia.

From each gland a duct is given off. The ducts from the anterior glands pass backwards, at the same time dipping down towards the ventral surface. Those from the posterior pair are much shorter, and dip down, passing forwards to meet and fuse with those from the anterior glands. From this point they continue in their ventral course as single ducts, one on each side, closely embracing the nervous system. These two ducts eventually meet and join in the median ventral line, and then proceed in an anterior direction as a single duct, which lies directly beneath the sub-oesophageal ganglion (see Plate I, Figs. 17, 18 and 19). This main duct ultimately opens near the tip of that part of the labium which forms the small under-lip. As has been stated before, *S. rosarum* has no salivary pump to force the secretion of the salivary glands into the wound made by the setae, and, as it does not form galls, it is probable that the salivary secretion is used to prevent the sap which oozes from the wound in the host-plant coagulating when it comes into contact with the atmosphere. The way in which the salivary secretion is used is probably as follows. The saliva, when the glands are active, will be forced out of the aperture in the small under-lip by the pressure behind of the newly-formed secretion, and perhaps by some contraction of the main salivary duct. It will be remembered that the under-lip is closely applied to the base of the beak or proboscis, and the secretion being poured out from it would naturally run down the groove which traverses the length of the upper surface of the beak. Consequently, it would meet and mix with the sap oozing from the wound made by the setae, and the mixture of the two would then be sucked up into the pharynx and thence into the crop. It is quite possible, therefore, that the salivary secretion, besides preventing the coagulation of the sap, may contain some digestive ferment to assist in digestion.

The salivary glands are composed of very large cells—especially the posterior pair—with closely granular cell contents and large nuclei with well-defined nuclear bodies.

In the posterior pair, the boundaries of the individual cells separate as they pass from the exterior to the interior, leaving spaces between

them, and these presumably form channels to conduct the secretion to the duct which leaves the gland (Plate I, Fig. 16). In the anterior pair this is not so marked.

The Nervous System.

The nervous system of the Aphides was for a long while undescribed. Buckton (1875—1882) in his monograph, entirely omits to figure the central nervous system, as such. Witlaczil (1882) gives figures of the central nervous system of *Aphis pelargonii* and *Pemphigius spirothecae.* These have been very useful for comparison with that of *S. rosarum.* Mordwilko (1895) also gives an account of the nervous system of *Trama* and *Lachnus.*

The *central nervous system* of *S. rosarum,* in common with the Aphides in general (as Witlaczil (1882) has shown), is, compared with that of the typical insect, greatly foreshortened, and considerable fusion and elimination of ganglia have taken place; and often it is only the distribution of the nerves which gives a clue to the identity of the ganglia.

The central nervous system (Plate I, Fig. 20) consists of a series of ganglia comprising two cerebral or supra-oesophageal ganglia, each with its optic lobe or ganglion; two sub-oesophageal ganglia; three pairs of thoracic ganglia; and a single median abdominal ganglion. The cerebral ganglia are placed in the head and are joined to the sub-oesophageal ganglia by the usual circum-oesophageal connectives, which in this case are short and stout. The sub-oesophageal ganglia are connected with the first pair of thoracic ganglia by what seems to represent a remnant of a primitive double nerve-chain.

The three pairs of thoracic ganglia, and the median abdominal ganglion, are massed up together, and lie in a position between the insertions of the first and second pairs of legs.

The ganglia give off nerves which pass to their respective destinations.

The two *cerebral ganglia,* each bearing an optic lobe, occupy nearly the entire cavity of the head. The ganglia themselves are somewhat ovoid, or, better, pyriform in shape, the expanded end being directed towards the dorsal surface of the head, and the more attenuated end towards the ventral side. From the outer lateral surface of each cerebral ganglion arises, partly from the attenuated end, and partly from the lower portion of the expanded end, an optic lobe, which, seen

from above, appears nearly as large as the cerebral ganglion to which it belongs. Each cerebral ganglion gives off a nerve to the antenna of its side, and a short stout nerve is given off from each optic lobe to the eye and ocellus to which it is adjacent. From the lower and smaller portion of each of these two cerebral ganglia arises a short circum-oesophageal connective, joining them up with the sub-oesophageal ganglia, and, through the loop thus formed, the oesophagus passes in its course from the mouth.

From each sub-oesophageal ganglion a nerve is given off which passes forward and is lost among the maze of muscles connected with the mouth parts.

Extending from the sub-œsophageal ganglion is an elongated portion which, as already suggested, probably represents the anterior portion of a primitive double nerve-chain. Joined to this elongated portion is the ovoid mass of nervous material mentioned previously as being made up of three pairs of thoracic ganglia and a single median abdominal ganglion. Each of the paired thoracic ganglia gives off a nerve to its respective leg, and each nerve appears to bifurcate just previous to its entry into the leg. From the abdominal ganglion a

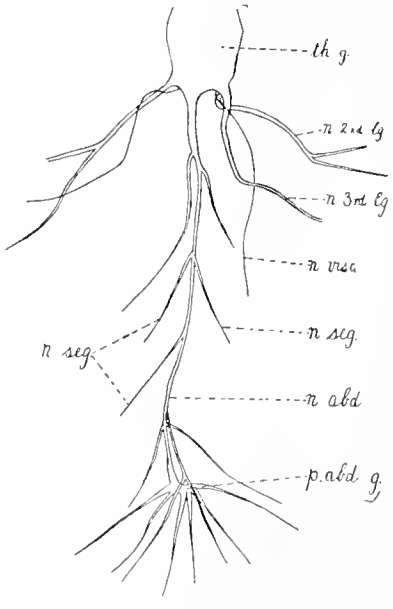

Fig. 12. The distribution of the nerves from the thoracic ganglia, and the median abdominal ganglion. (× 54.)

n. 2nd and *3rd lg.* = nerves to the 2nd and 3rd legs. *n. abd.* = abdominal nerve.
n. seg. = nerves to the abdominal segments. *n. visc.* = nerve to the viscera.
p. abd. g. = posterior abdominal ganglion. *th. g.* = thoracic ganglia.

stout nerve is given off which extends in the median line to the posterior end of the body. There it expands into a fairly large ganglion from which nerves are given off, which, from their position, must pass to the muscles which control the anal and reproductive apertures. Also nerves are given off from the main trunk during its passage from the median abdominal ganglion to the posterior abdominal ganglion. These nerves are given off fairly regularly in pairs and pass to the segments of the abdomen.

Text Fig. 12 is a camera lucida drawing of a dissection showing this abdominal nerve and the posterior abdominal ganglion with the nerves they give off. In addition to this abdominal nerve, two small nerves are given off from the median abdominal ganglion one on either side of the above-mentioned nerve, and it is to be inferred from their course that they pass to the viscera.

The Tracheal System.

The tracheal system almost completely eluded the efforts of the early investigators like Dufour to figure it with anything approaching completeness.

From the literature I have been able to obtain, it seems that Witlaczil was among the first to give illustrations of the respiratory apparatus of Aphides. He figures the tracheal system of *Aphis pelargonii* (1882), and also indicates the general tracheal system of a *Coccid*, and the apterous stage of *Chermes abietis* and *Phylloxera quercus* (1886). The results I have obtained agree very largely with his representation of *Aphis pelargonii*. Flögel (1904 and 1905) also describes the tracheal system of the winged female of *Aphis ribis*, but no figures are given.

Notwithstanding the small size and somewhat sedentary habit of the Aphis in this stage of its life history, the tracheal system is developed to a remarkable degree (Text Figs. 13 and 14).

Situated along the sides of the body are nine pairs of stigmata—two pairs being thoracic and the remaining seven abdominal. The first pair is placed in the posterior margin of the first thoracic segment, and the second pair on the anterior margin of the third thoracic segment. The second thoracic segment is entirely destitute of stigmata. The seven pairs of abdominal stigmata are situated—one pair in each—in the first seven segments of the abdomen. The tracheal system is composed of well-defined dorsal and ventral systems—a short trachea

arising from each stigma and dividing into dorsal and ventral branches (Plate I, Figs. 22 and 23).

The *dorsal tracheal system.* The dorsal branches (Text Fig. 13), when given off from the main trachea, pass along under the latero-dorsal surface of the body to a position about two-thirds of the way between the lateral and the dorso-medial lines. In this position, each

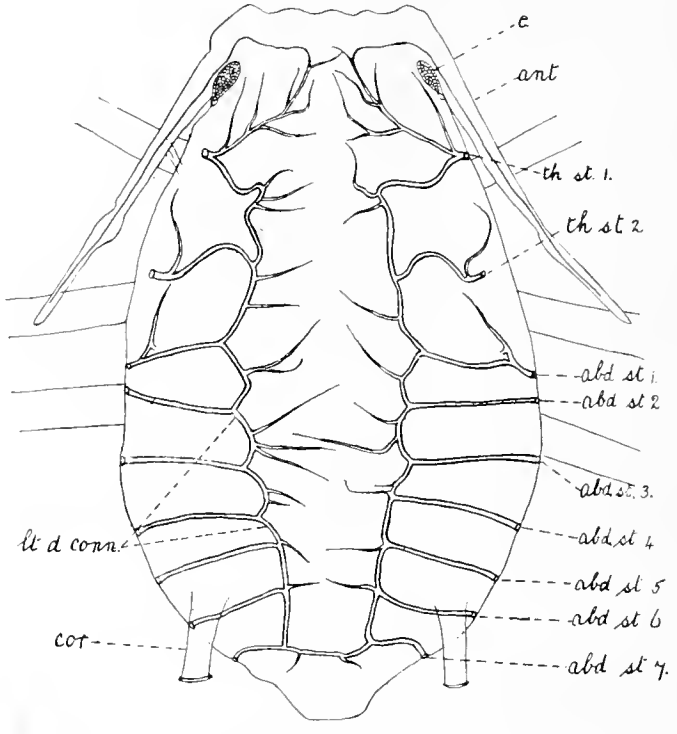

Fig. 13. The Dorsal Tracheal System. (×46.)

abd. st. 1—7 = stigmata of the 1st—7th abdominal segments. *ant.* = antenna. *cor.* = cornicle. *e.* = eye. *lt. d. conn.* = left dorsal connecting trachea. *th. st.* 1 and 2 = 1st and 2nd thoracic stigmata.

branch, with the exception of the first thoracic, divides again into two, and the branches join up to form a single longitudinal trachea (*d. conn.*) (the Längsstämme of Witlaczil) on each side of the dorso-medial line, extending from the first thoracic segment to the seventh abdominal segment. The course followed by this longitudinal connective trachea is a zigzag one, owing to the subdivisions of the dorsal tracheae meeting at an angle. From each of the long tracheae—one on each side of the median dorsal line—small ramifications are given off into the space enclosed by them. These branches pass downwards and aerate the organs in their immediate vicinity. The right and left divisions of

the dorsal system thus formed by the anastomosis of these branches, are not connected with one another except in the seventh abdominal segment, where the posterior branches formed by the bifurcation of the seventh dorsal tracheae meet in the middle line, forming a connection between the two lateral systems.

The *first thoracic stigmata.* From the first thoracic stigma on each side, in addition to the dorsal trachea which joins the long connective, a large branch is given off near the commencement of the main trachea. This extends in an oblique direction towards the median line of the head, and during its course gives off smaller branches to the surrounding parts. Having arrived at a position opposite to the base of the antenna, it turns abruptly, passes to the insertion of the antenna, and continues along it nearly to its extremity.

The *second thoracic stigmata.* A lateral branch is given off near the beginning of the dorsal trachea arising from the second thoracic stigma, but this does not give off any supplementary branches.

The *abdominal stigmata.* Near the commencement of the dorsal trachea which proceeds from the first abdominal stigma on each side, a small branch is given off which extends in an anterior direction. From this the ventral branch in this case originates; in all other cases it is given off from the main trachea proceeding from the stigma. From the sixth dorsal branch on each side, a small trachea is given off to the cornicle, to which it is adjacent. This is not shown in Text Fig. 13, because for some reason it had become invisible in the specimen from which that drawing was taken.

The ventral tracheal system. In this system (Text Fig. 14) it is only the ventral branches given off from the abdominal stigmata which are connected up by the longitudinal trachea. Those from the thoracic stigmata are not so connected. The fusion is effected in exactly the same way as in the case of the dorsal system—the ventral branches, with the exception of the first, dividing when they have reached a position about half way towards the ventral median line, the branches so formed joining up to constitute the long connecting trachea, which, in this case, will only extend from the first to the seventh abdominal segment.

As in the case of the dorsal system, small tracheae are given off from the long connectives into the central space. These dip down and aerate the tissues in their immediate vicinity.

Another feature in which the ventral differs from the dorsal system is that each of the main ventral branches from the opposite thoracic stigmata

meets and fuses in the middle line with its fellow of the opposite side, so that each pair of thoracic stigmata are connected by a large trachea crossing the thorax transversely. From these tracheae branches are given off to the various parts of the thorax.

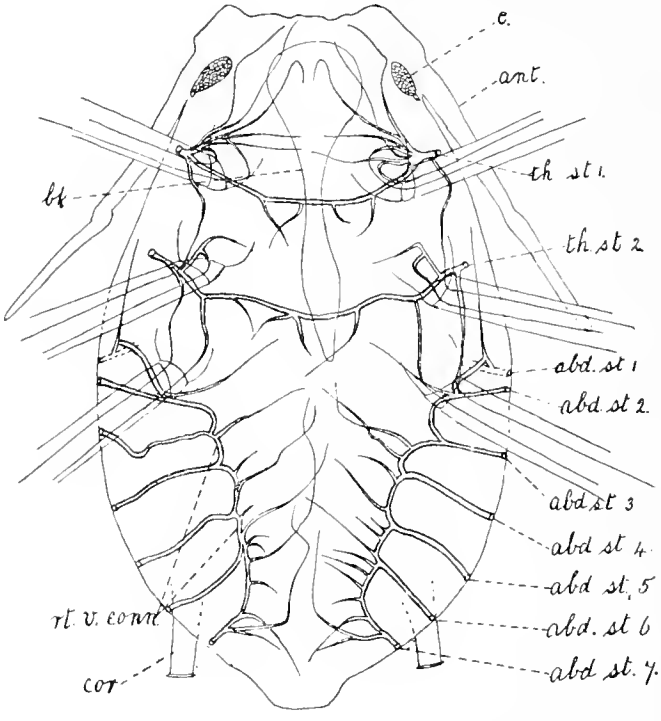

Fig. 14. The Ventral Tracheal System. (×46.)

abd. st. 1—7 = stigmata of the 1st—7th abdominal segments. *ant.* = antenna.
bk. = beak or proboscis. *cor.* = cornicle. *e.* = eye.
rt. v. conn. = right ventral connecting trachea.
th. st. 1 and 2 = 1st and 2nd thoracic stigmata.

The *first thoracic stigmata.* From the trachea which connects the first pair of thoracic stigmata, a branch arises on each side near to the stigmata, passes backwards, and enters the second leg of its side. This branch extends to the end of the leg. Two other small branches pass off in the central region towards the hinder border of the first thoracic segment.

A short distance along the main transverse trachea, five branches arise close together on each side, and pass anteriorly. The two outermost ones pass to the front of the head; the next one to the base of the proboscis or beak; and the two innermost ones to the central region of the head. The anterior one of the last-mentioned tracheae approaches

its fellow of the opposite side but no distinct fusion takes place between them.

The two tracheae which pass to each of the first pair of legs arise from the last but two and last of these branches—the most anterior one passing to the end of the leg, and the other continuing only to the end of the femur.

The *second thoracic stigmata.* Near the commencement of the main ventral transverse trachea arising from the second thoracic stigma on each side, a branch is given off which passes directly posteriorly and enters the third leg.

This trachea traverses the leg to its extremity. A little farther along the main trachea, another branch is given off in an anterior direction; from this a sub-branch arises, which turns posteriorly and enters the second leg. In the latter case, however, the trachea only extends to the end of the femur. Still a little farther along the main trachea another branch is given off which passes directly backwards and enters the third leg, but only continues to the end of the femur. The remaining branches from this transverse trachea connecting the second pair of thoracic stigmata, pass to the region traversed by it.

The distribution of the branches to the legs yields reliable evidence —apart from their position—that the two pairs of thoracic stigmata belong to the first and third thoracic segments—each thoracic stigma contributing one branch for the aeration of the second pair of legs to take the place of the tracheae which would arise from the stigmata, were they present, of the second thoracic segment.

The Reproductive System.

The reproduction of the Aphides in this stage of their life history has attracted a great deal of attention from the fact that it exhibits the peculiarity of parthenogenetic reproduction. This being so, there is abundant literature on the subject, but it all—or nearly all—deals with the actual segmentation of the eggs, or with the nuclear changes thus involved.

The morphology of the reproductive system is so simple that the figures given by the earliest investigators still remain almost unaltered to-day.

Dufour (1833), Buckton (1875—1882), Witlaczil (1882) and others give accounts and illustrations of the appearance of this system.

The *reproductive system* (Text Fig. 15) offers no very remarkable

features from a morphological point of view. It consists of what has been termed a pseudovarium, which is made up of a series of egg tubes arranged in two groups—one on each side of the body.

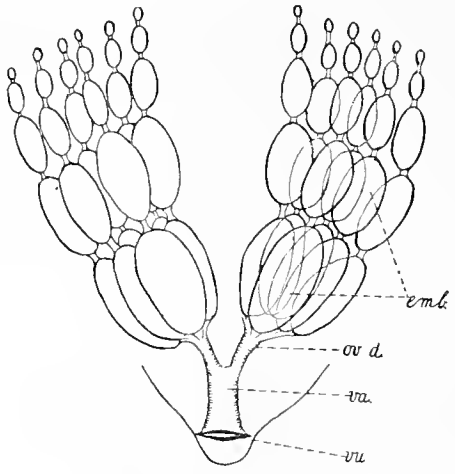

Fig. 15. A dorsal view of the reproductive system. Semi-diagrammatic. (×35.)
emb. = embryos. *ov. d.* = oviduct. *va.* = vagina. *vu.* = vulva.

The individual tubes of each group meet at their posterior ends, and each opens into a single tube or oviduct. The two oviducts—one from each group—join at the middle line to form a single wide canal—the vagina—which extends to the external aperture—the vulva—situated at the posterior end of the body, just below the anus.

As the Aphis reproduces its kind parthenogenetically and viviparously in this stage of its life history, the egg tubes contain embryos in varying stages of development. As Dufour and others have observed, the embryos always develop with their posterior ends pointing towards the mouth of the tube; consequently they are always born posterior end first.

The two oviducts and the median vagina lie obliquely—dorso-ventrally—in the body. As it passes from the vulva, the vagina curves upwards until it lies just beneath the rectum. Here it divides to form the two oviducts, the whole taking the form of a letter Y, in the fork of which the rectum lies. The oviducts continue their upward course—the egg tubes lying mainly above the alimentary canal.

The "Secundäre Dotter."

The above is the name given by the German investigators to the masses of granular material found in the body cavity of the Aphis and

lying between the egg-tubes. It has also been termed the "Pseudo-vitellus." I have been unable to determine any definite distribution for it, and the quantity varies in different specimens.

Structurally, it is made up of very large cells (Plate I, Fig. 24) with well-defined nuclei, and the granular cell contents resemble very greatly the yolk of an ordinary insect egg.

Witlaczil (1882) attaches an excretory function to it, but both from its position and structure it is more likely to be nutritive in function.

Circulatory System.

I have been unable to find any structure which would answer to a dorsal vessel or heart in *S. rosarum,* and it is probable that any blood system which may be present consists of a series of haemocoeles.

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REFERENCE LETTERING.

abd. g.	Median abdominal ganglion.
ams. v.	Arms of the inverted V.
an.	Anus.
ant. lat. r.	Anterior lateral rod.
b. se.	Bases of the setae.
bk.	Beak or proboscis.
c. g.	Cerebral ganglion.
ch.	Chitin.
ch. r.	Chitinous rods attached to the lower parts (*j*) of the arms of the Λ, and joined to the bases of the setae.
cir. oes. conn.	Circum-oesophageal connectives.
circ. mus.	Circular muscle fibres.
crp.	Crop.
d. br.	Dorsal branch of trachea.
d. conn.	Dorsal connecting trachea.
e.	Eye.
el. m.	Muscles stretched between the transverse bar and the roof of the head and which serve to lift up the transverse bar after some displacement.
emb.	Embryo.
epi.	Epidermis.
fb.	Fat body.
gr.	Granules.
j.	Jointed lower parts of the arms of the Λ.
l. pr. m.	Large protractor muscle.
lbr.	Labrum.
lm.	Labium.
long. mus.	Longitudinal muscle fibres.
m. ph.	Muscles for dilating the pharynx.
mus.	Muscles.
n. abd.	Abdominal nerve.
n. ant.	Nerves to the antenna.
n. e.	Nerve to the eye.
n. lg. 1, 2, 3.	Nerve to the 1st, 2nd and 3rd legs.
n. mus.	Nerve to muscles controlling mouth organs.
n. visc.	Nerve to the viscera.
nu.	Nuclei.
nu. b.	Nuclear bodies.
oes.	Oesophagus.
op. l.	Optic lobe.
ov. d.	Oviduct.

p. m.	Small protractor muscle of the posterior setae.
ph.	Pharynx.
r. m.	Retractor muscles of the setae.
sal. gl. ant.	Anterior salivary glands.
sal. gl. d.	Duct of salivary glands.
sal. gl. post.	Posterior salivary glands.
se.	Setae.
st.	Stigma.
sub. oes. g.	Sub-oesophageal ganglion.
th. g. 1, 2, 3.	1st, 2nd and 3rd thoracic ganglia.
tr. b.	Transverse bar.
u.	U-shaped piece of chitin which serves as the origin for the large protractor muscle.
va.	Vagina.
v. br.	Ventral branch of trachea.
v. conn.	Ventral connecting trachea.
vu.	Vulva.

EXPLANATION OF PLATE I.

All drawings were made with the camera lucida unless otherwise stated.

Fig. 1. General frontal view of chitinous framework after preparation with caustic potash to clear away all the muscles. (× 230.)

Fig. 2. Transverse section across the tip of the labrum to show the setae cut across. (× 1050.)

Fig. 3. Longitudinal horizontal section across the lower part of the head to show the chitinous rods joining on to the bases of the setae. (× 355.)

Fig. 4. Longitudinal vertical section through the head to include one end of the transverse bar. (× 108.)

Fig. 5. Transverse section through the head to show the course of the large protractor muscle. (× 108.)

Fig. 6. The insertion of the large protractor muscle into the transverse bar. (× 355.)

Fig. 7. Origin of the large protractor muscle. (× 630.)

Fig. 8. Transverse section through the oesophagus. (× 630.)

Fig. 9. Transverse section through the crop. (× 315.)

Fig. 10. Transverse section through the coiled intestine. (× 315.)

Fig. 11. Transverse section through the rectum. (× 315.)

Fig. 12. Median longitudinal vertical section through the head to show the pharynx and the muscles which dilate it. (× 108.)

Fig. 13. Longitudinal section through the crop to show the insertion of the oesophagus and the post-mortem appearance of the contents. (× 355.)

Fig. 14. Longitudinal section through a part of the rectum to show the muscle fibres in the wall. (× 355.)

Fig. 15. Longitudinal section through an anterior salivary gland. (× 630.)

Fig. 16. Longitudinal section through a posterior salivary gland. (× 630.)

Fig. 17. Transverse section through the body in the region of the posterior salivary gland to show the duct from the gland passing round the nervous system to the mid-ventral line. (× 152.)

Fig. 18. The next serial section to that shown in Fig. 17. In this section the main salivary duct is seen in the mid-ventral line beneath the nervous system. (×152.)

Fig. 19. A longitudinal oblique section through the middle region of the body, showing the two salivary glands of one side, the duct from each meeting and joining, and the main salivary duct beneath the nervous system. (×236.)

Fig. 20. The central nervous system as seen from above. Semi-diagrammatic. (×152.)

Fig. 21. Longitudinal horizontal section cutting through an embryo just about to descend through the oviduct into the vagina and pass outwards. (×72.)

Fig. 22. Lateral view of the two stigmata to show the dorsal and ventral divisions of the short main trachea from the stigma. (×108.)

Fig. 23. A single stigma to show short main trachea. (×630.)

Fig. 24. A piece of the "secundäre Dotter." (×355.)

Corrections: In Fig. 1 the chitinous rods (*ch. r.*) on the left side of the figure should show a cross line as on the right above the upper dotted line leading to *ch. r.* In Fig. 4 the dotted line leading from *p. m.* (small protractor muscle) should not traverse the space to the right of the muscle. In Fig. 20 for *circ. ves. conn.* read *circ. oes. conn.*; the swelling beneath (opposite *n. mus.*) represents the sub-oesophageal ganglion.

NOTE.

Since going to press, it has been brought to my notice that the homologies of the Hemipterous mouth parts are now generally accepted. The structures which have been called the anterior setae will therefore represent the mandibles, and the posterior setae, the first maxillae.

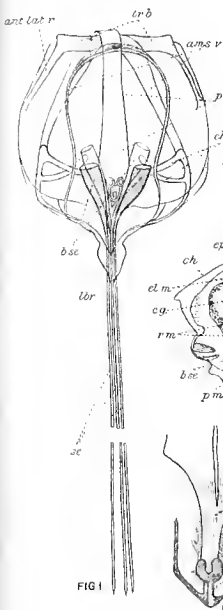


FIG 1

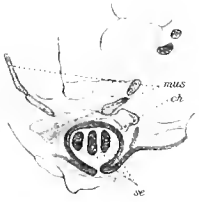


FIG.2.

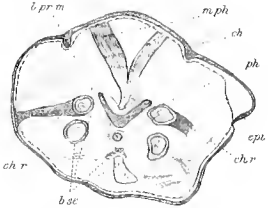


FIG 3

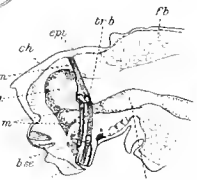


FIG 4

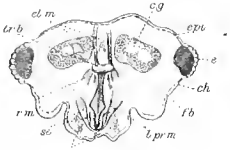


FIG.5.

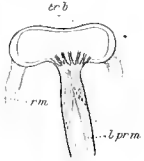


FIG 6.

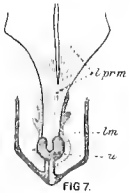


FIG 7.

FIG.8

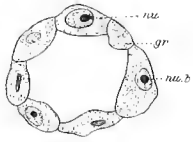


FIG.9

FIG 10

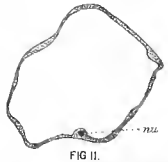


FIG 11.

FIG.14

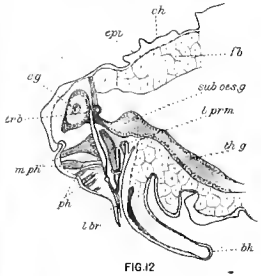


FIG.12

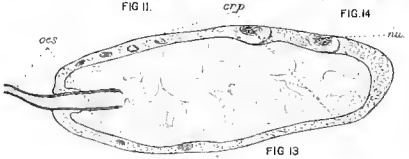


FIG 13

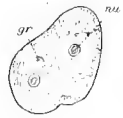


FIG 15

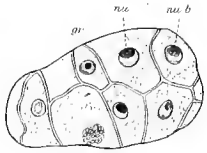


FIG 16

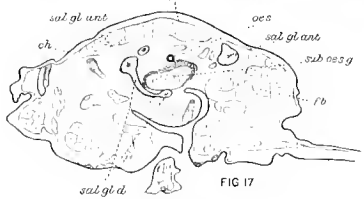


FIG 17

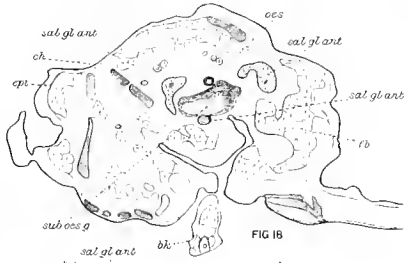


FIG 18

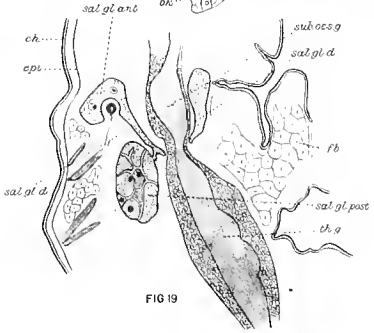


FIG 19

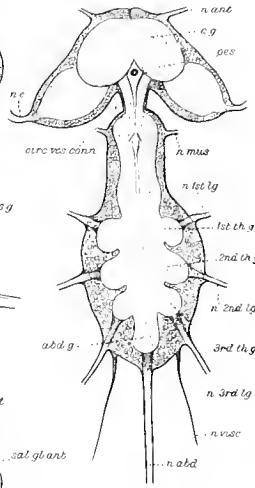


FIG 20

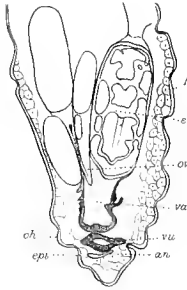


FIG 21

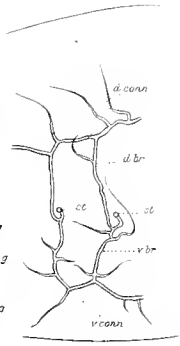


FIG.22

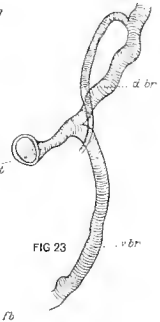


FIG 23

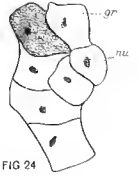


FIG 24

A.J Grove, del

E. Wilson, Cambridge

FATAL ENTERITIS IN A TIGER CAUSED BY *PHYSALOPTERA PRAEPUTIALIS.*

By A. J. HARRISON, M.B.

Consulting Physician to the Bristol General Hospital,

and I. WALKER HALL, M.D.,

Professor of Pathology, University College, Bristol, and Pathologist to the Royal Infirmary, Bristol.

A male Malay tiger was brought to the Clifton Zoological Gardens in July, 1908. He was then very thin and fed badly. After a time he settled down in his new quarters, and ate his food with greater relish. He appeared to be in good health and his coat was sleek and fine, but he never jumped or played in the usual way of his species. On February 4th he vomited, and had a severe attack of diarrhoea. His breathing became less abdominal and more thoracic in character, and death occurred two days later.

The post-mortem examination showed that the animal had been well nourished and that death was due to enteritis caused by round worms. Owing to the kindness of Messrs A. E. Shipley and R. T. Leiper the causal parasite has been identified as *Physaloptera praeputialis.* This nematode has been fully described by von Linstow (1888).

Autopsy.

On opening the abdominal cavity, a round worm, measuring about $1\frac{1}{2}$ inches by 1/8-inch, was seen coiled up just under the serous capsule of the liver. Calcified areas, corresponding in size to that of the parasite mentioned, were present in the subcapsular tissues of the spleen. Under the serous coat of the stomach, intestine and colon, a number of well-marked swellings were seen; these were arranged irregularly and varied considerably in size and shape. Some of them

formed a segment of a circle, others were cone-shaped, others again had the appearance of an elongated cone and at the tip of the cone there was sometimes a "pin-point" orifice. All the swellings were uniformly hard and anaemic; their borders were well-defined, and they appeared to be seated in the muscular structures. The overlying serosa was not the seat of any exudative process; there was a little thickening of the subendothelial tissues but there were no signs of adhesions. The process showed the signs of chronicity and was apparently enterogenous in origin.

When the stomach and intestines were opened up, similar nodules projected into the lumen of the canal. In this case they were covered by the mucosa; at or about the centre of the raised area, however, the mucous coat was deficient, leaving an aperture which communicated with the interior of the nodule.

In the stomach the nodules were few in number but large in size; in the small intestines they were more numerous, of medium size, and subserous rather than submucous; in the large intestine there were so many that they practically occupied the entire area, being separated from one another by a small area of lymphoid tissue only.

The whole alimentary tract was in a condition of advanced catarrhal inflammation, and in the lumen large numbers of the worms were present.

When the nodules were bisected, they were found to consist of dense fibrous tissue surrounding an irregularly branching lumen. In the lumen a number of small worms were seen. Microscopical examination showed that each of the young worms was surrounded by bands of very dense fibrous tissue, and that the fibrosis was less marked towards the periphery of the mass; the peri-nodal tissues showed but slight proliferative changes.

The general appearances suggested that the female worm burrowed through the mucosa and deposited embryos in the submucosal tissues. During the development of the worms a fibrous nodule was formed, and the nodule extended into the muscular layer causing considerable atrophy of the muscle fibres, and in some cases reached the subserous tissues. In the latter instance, when the young worm happened to lie immediately beneath the serosa or was cut off from the intestinal aperture by fibrous tissue, it commenced to burrow and so reached intraperitoneal organs, such as the liver and spleen. When, however, the worm was in a position to utilize the opening into the alimentary canal it emerged from the nodule, buried its head in mucosal tissue and

so commenced its enterogenous parasitic life. These lesions are somewhat similar to those described by von Linstow (1879).

The widespread distribution of the worms and the extensive chronic irritation of the gastric and intestinal wall were quite sufficient to account for the death of the animal.

The lungs were riddled with cysts containing the *Paragonimus westermani* (Kerb); there was but little peri-cystic consolidation or signs of marked interference with pulmonary functions. The other organs were normal.

REFERENCES.

Von Linstow (1879). *Archiv für Naturgeschichte*, p. 181.
—— (1888). *Ibid.* p. 239.

AMOEBA CHIRONOMI, NOV. SP., PARASITIC IN THE ALIMENTARY TRACT OF THE LARVA OF A *CHIRONOMUS*.

By ANNIE PORTER, B.Sc. Lond.,

Zoological Research Laboratory, University College, London.

(With 21 Text Figures.)

The occurrence of an *Amoeba* in the alimentary tract of the familiar "blood-worm" or *Chironomus*-larva has not been recorded before so far as I am aware. Inasmuch as locomotion is effected by means of a pseudopodium lying in a definite direction, and as the motion is very rapid for a parasite of its class, the *Amoeba* possesses special interest.

Materials and Methods.

The investigation of this Amoeba, which I call *Amoeba chironomi*, n. sp., was conducted during November and December, 1908, and January, 1909, when material was not easily obtainable. The *Chironomus*-larvae in which these interesting Protozoa occurred were young forms not more than half grown, and were actively engaged in tube formation, using the *Spirogyra* and *Elodea* in the vessel in which they were kept, as a basis for the building of their tubes. All the larvae were very active. Of those examined about half were found to be infected, but in no case was the infection a very heavy one.

For purposes of examination, the gut of the larva was rapidly removed and placed on a glass slide together with physiological salt solution at the temperature of the water in which the *Chironomi* had been living. Fresh preparations were so examined. Methylene blue was used as an *intra vitam* stain, and was found of service, particularly in observing the contractile vacuole. Encystment was seen

to occur readily when the salt solution in the preparation became concentrated. For observing the living organism, preparations of the teased gut were generally used.

Various methods of fixation were employed, but whether osmic acid vapour, or absolute alcohol were employed, fixation almost always had the effect of causing instantaneous withdrawal of any pseudopodia, and the assumption of the encystment form. Corrosive sublimate-alcohol was better for preserving the true form of the organism. The protoplasm of these *Amoebae* seemed remarkably sensitive, and apparently they live in a most intimate, harmonic, osmotic relation with the intestinal fluid of their host. Very slight changes of temperature or concentration of solution outside the gut, as on the slide or cover-slip, results in encystment. The condition that produces this result within the gut of the host is probably the concentration of the gut contents as they near the anal region. That this is the case is certain, for the posterior one-sixth of the gut was seen to contain semi-solid contents and among these the cysts were found, and voided excrement also contained cysts of the *Amoeba*.

Occurrence of the Parasites in the Host.

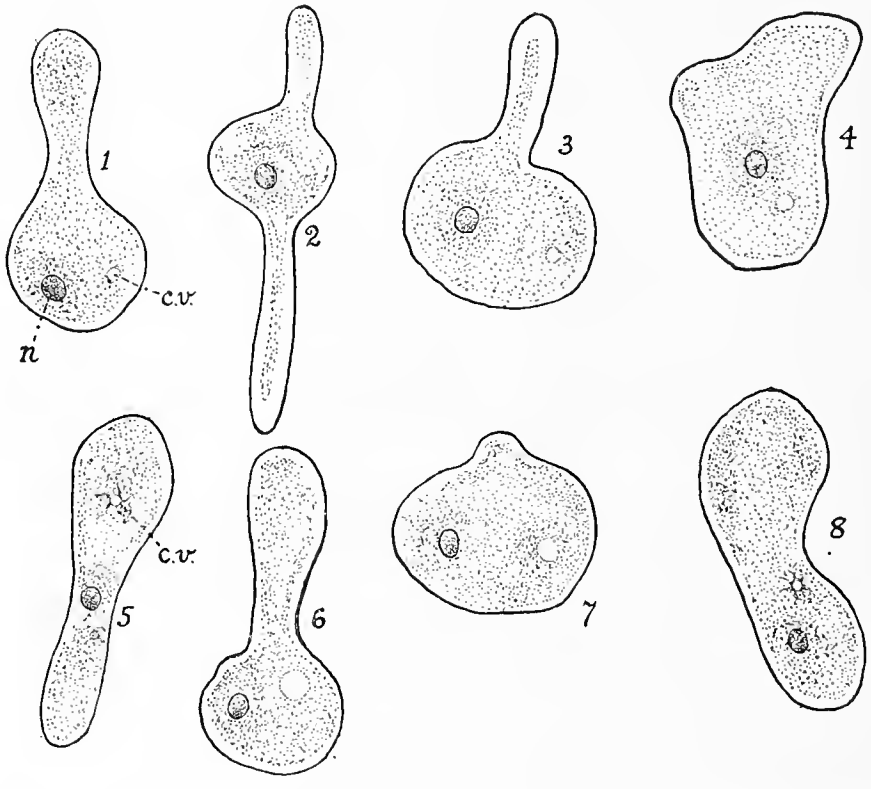
The *Amoebae* were first noticed in the ooze from the ruptured gut of a recently killed larva. An unruptured gut was then carefully examined and the parasites could be seen within, making their way about. They occur throughout the gut, except just near the mouth. The posterior one-sixth of the gut (rectum) contained very few moving forms, but cysts were present, mingled with faecal matter. Examination of the faeces showed that cysts of the *Amoeba* were present in them and the cysts were also found in the "castings" in the tubes of the larvae of the *Chironomus*.

The parasites can be easily distinguished from the lymphocytes and wandering cells so common in insect larvae, for the *Amoebae* possess a well marked, contractile vacuole.

General Description.

The organism in life varies considerably in appearance at different times according to its various phases of movement (Figs. 1—15). The body of the parasite consists of an elongate, oval mass of protoplasm in which a nucleus and contractile vacuole can be seen (Figs. 6, 7). The

nucleus appears as a clear, refractile, vesicular mass. The general protoplasm is divisible into a clearer ectoplasm and a granular endoplasm (Figs. 2, 3, etc.). Pseudopodia are formed during movements of locomotion by an outflow of the ectoplasm at right angles to the long axis of the body of the *Amoeba* (Figs. 3, 10, 11, 12). Into this ectoplasmic process the granular endoplasm streams and soon becomes concentrated there (Fig. 16). The pseudopodium then swells out and the *Amoeba*



Figs. 1—15. Drawings, showing variations in shape and reversal of direction of motion of an *Amoeba chironomi*, made from a single living specimen during a period of 45 minutes, at intervals of approximately 3 minutes.

Fig. 1. *A. chironomi* with somewhat broad, blunt pseudopodium partially withdrawn. Contractile vacuole (*c.v.*) expanded. Nucleus (*n*) present.

Fig. 2. Long pseudopodium forming before previous one is entirely withdrawn. The spindle shape of the organism is the result of change of direction of motion.

Fig. 3. Oval shape, with single pseudopodium, where the clear ectoplasm is well seen in contrast with the granular endoplasm.

Fig. 4. Disappearance of pseudopodium.

Fig. 5. Contractile vacuole (*c.v.*) partly contracted.

Fig. 6. Contractile vacuole widely expanded again.

Fig. 7. Shape of organism now almost oval.

Fig. 8. Contractile vacuole reduced in size again.

rapidly becomes oval again. As the medium in which the organism is being examined under the microscope gradually becomes denser, thus placing the *Amoeba* in an unfavourable environment, the movements slow, the organism ceases to form pseudopodia, and encystment ensues (Figs. 18—21). I have watched the whole process of encystment proceed on a slide under the microscope.

Movements.

The movements of *Amoeba chironomi* are active, a somewhat unusual occurrence among organisms of this class. Continuous progression in one direction may take place, or reversal of the direction of

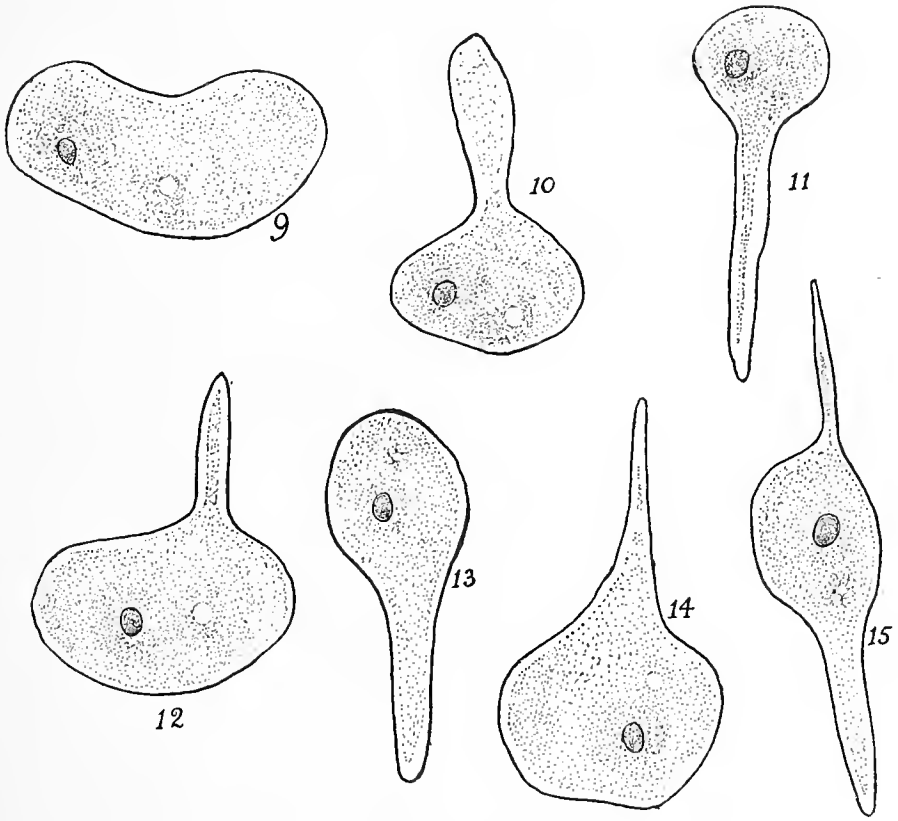


Fig. 9. Organism extended and very much flattened.
Fig. 10. New pseudopodium protruded in a forward direction.
Fig. 11. Direction of motion reversed. A backwardly directed filopodium now present.
Figs. 12, 13, 14. Successive reversals of direction of movement.
Fig. 15. Formation of large backwardly directed pseudopodium before previous one (forwardly directed) is completely withdrawn. Beginning of further reversal of direction of motion. Cf. Fig. 2.

motion may occur. These movements may be considered separately; in each case, the movements are brought about by the agency of pseudopodia. Pseudopodia are formed by the very rapid protrusion of the ectoplasm, and the process so formed remains clear and transparent for a short time, its shape being more or less conical, sometimes more of the nature of a filopodium than of a lobopodium (Figs. 11, 14). The richly granular endoplasm, which up till now has exhibited peripheral streaming movements, then flows forwards into the ectoplasmic process, but remains surrounded by a thin layer of hyaline protoplasm (Figs. 3, 11). In continuous progression in one direction, the ectoplasm at the apex of the pseudopodium, under the forward pressure of the endoplasm, extends laterally, and the rest of the body of the organism gradually flows into the swollen pseudopodium, and so an oval body is produced, the long axis of which is at right angles to the original pseudopodium (Figs. 6, 7). Almost as soon as this oval form has been assumed, a new process arises in relatively the same region of the body as did its predecessor. At first, the process is purely ectoplasmic and conical; then inflowing of endoplasm occurs and finally the shape of the organism becomes oval again. So far as I could determine there was little or no interval between the assumption of the oval form and the formation of a new pseudopodium; that is, the formation of a new pseudopodium begins directly after the disappearance of the previous one. The short axis of the oval form was always the axis about a continuation of which the pseudopodium was produced.

Reversal of the direction of movement is fairly common. It is brought about by the formation of a pseudopodium at the end of the organism opposite to that at which the pseudopodium for forward progression is produced (Figs. 1, 2 and 14, 15). Frequently the organism begins to reverse its movement before the forwardly directed pseudopodium is completely withdrawn. In other words, the ectoplasmic portion of the original pseudopodium may persist for some time, with the result that the organism is temporarily spindle-shaped, with a somewhat globular, richly granular body and a rather clear pseudopodium at either end (Figs. 2, 15). The ectoplasm of the original pseudopodium is gradually withdrawn from the previously forwardly directed end, and then the streaming of the endoplasm into the second ectoplasmic process initiates active movement in the reverse direction.

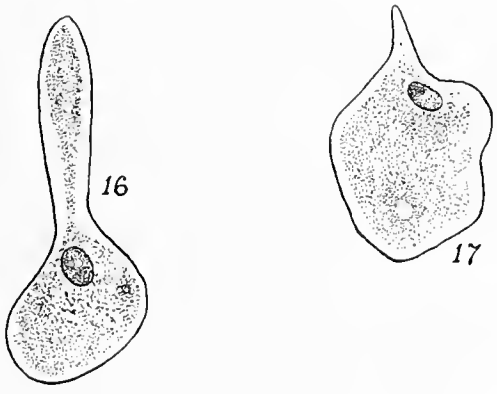
In fresh preparations, I noticed that the *Amoebae* kept in the neighbourhood of the tissue of the gut wall and rarely migrated far from it. In unruptured intestines the *Amoebae* could be seen moving

rapidly over the gut wall and also over the surface of the food column, never within it. I have not seen them mingled with the semi-digested fluid in the centre of the lumen of the gut.

Morphology.

Amoeba chironomi is very difficult to measure, since its shape varies, while it does not always extend itself exactly in the plane of the field of observation, and so at times appears thicker than at others. However, the bodies of the *Amoebae* which I have seen are from 15μ to 18μ long and 10μ to 12μ wide, exclusive of pseudopodia. A pseudopodium may measure up to 15μ in length. Other forms may be found by subsequent observers with different dimensions from these. I will now deal with the morphology of the various parts and organellae of *Amoeba chironomi*.

Ectoplasm. This is remarkably well seen in the process of formation of pseudopodia, when an outflowing of clear, homogeneous ectoplasm takes place. Neither in fresh preparations nor in stained specimens can any detailed structure be observed, but the ectoplasm is very refringent and shows well in life (Figs. 2, 3, 16, 17, 19).



Figs. 16, 17. Drawings of two separate *Amoeba chironomi*, made with the aid of the camera lucida, from fixed and stained preparations (corrosive sublimate-alcohol, Delafield's haematoxylin). Nucleus shown with nucleolus to one side. Note concentration of endoplasmic granules around nucleus and contractile vacuole. In Fig. 16, similar concentration of endoplasm at the end of the pseudopodium, which is about to become oval.

Endoplasm. The endoplasm is richly granular throughout, the granules being fairly evenly distributed, though slight concentration occurs around the nucleus and the contractile vacuole (Figs. 16, 17). In life, the pulsation of the contractile vacuole can be well seen. The excretory products make this vacuole more noticeable, as these products

are faintly reddish in hue. A nucleus is present in the endoplasm. Food vacuoles were very rarely seen.

Contractile Vacuole. The occurrence of a contractile vacuole is most unusual in parasitic *Amoebae*, but one is found in *A. chironomi*. The contractile vacuole (Figs. 1, 5, *c.v.*) or excretory organella has more resemblance to that structure as seen in *Paramoecium* than to that of the common *Amoeba*. There would seem to be a series of fine curved radiating slits or canaliculi having a superficial resemblance to portions of the iris diaphragm of a camera or microscope (Figs. 5, 8, 13, 15, 16). The canaliculi are so thread-like that the vacuole never appears stellate. The vacuole at one time is widely expanded and ringlike (Figs. 4, 6, 7, 10, 12). It gradually contracts, the lumen remaining circular (Figs. 2, 5), and finally is obliterated (Fig. 11). It then re-opens. When widely expanded the contents of the vacuole appear faintly reddish.

If the *Amoeba* be "fed" with methylene blue solution, part of this dye finds its way into the vacuole, whose purplish colouration then suggests that of polychrome methylene blue.

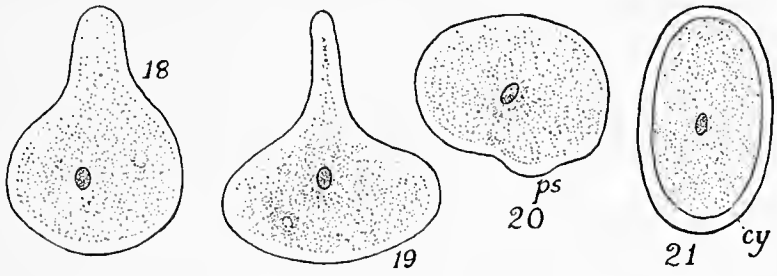
Nucleus. In life, the nucleus (Fig. 1, *n.*) is seen as a clear vesicle, but it is not particularly obvious in all specimens, being obscured by the very granular endoplasm. I had very little material for making stained preparations, but in the few I have, the nucleus is simple, is more or less oval in form, and shows a small, denser staining portion, the nucleolus (Figs. 16, 17) often to one side. There is little chromatin in the nucleus, but the nuclear membrane is sharply defined (Figs. 6, 16, 17). I regret that I have not definitely seen binary fission in *Amoeba chironomi*, and so have not found karyokinetic or other division figures in connection with the nucleus. This is probably due to the small amount of material at my disposal and to the season of the year (winter) when the work was done. One can hardly expect to find active multiplication of free forms in winter. Such division may occur at warmer periods of the year, or perhaps in older larvae of *Chironomus* than those examined.

Encystment.

Encystment of *Amoeba chironomi* can be induced in the laboratory by the simple method of allowing a preparation in physiological salt solution to become concentrated by slight evaporation. Under the influence of heat and increasing salinity, the protoplasm is unable to continue its

active movements (Figs. 18, 19). Pseudopodia are withdrawn and the *Amoeba* gradually becomes passive (Figs. 18, 19, 20). The streaming movements of the general protoplasm are slowed, and following this, the contractile vacuole suspends action (Fig. 20). The protoplasm then contracts around the nucleus and the organism appears as a small, oval, uninucleate body. Externally, a cyst wall, probably secreted by the ectoplasm, is formed (Fig. 21).

With one exception, the cysts I have observed are uninucleate. In one case, however, a fresh preparation was examined in which nuclear multiplication had occurred, five or six small, refractile daughter nuclei being present. I regret that I was unable to stain this particular cyst satisfactorily.



Figs. 18—21. Stages of encystment; drawn from a single living *A. chironomi* during a period of 45 minutes.

Figs. 18, 19. Progressive withdrawal of pseudopodium and closure of contractile vacuole.

Fig. 20. Organism has rotated. Contractile vacuole closed. The remaining small, blunt pseudopodium (*ps.*) took 20 minutes to withdraw entirely.

Fig. 21. Encysted *A. chironomi*; thick cyst wall (*cy.*) well seen.

The cyst wall is thick and refringent, of a chitinoid nature, and has the characters usually associated with cysts. There seem to be no spines, warts or markings upon the cyst wall. Encystment, as before mentioned, occurs naturally in the posterior one-sixth of the gut of the *Chironomus*, and is induced by the concentration of the intestinal contents. The natural cysts have precisely the same appearance as those artificially produced. They are from 12μ to 20μ long and 9μ to 12μ broad.

The time necessary for artificially induced encystment varies. I have once seen encystment take place in only two minutes, but in the form figured (Figs. 18—21) the entire process of cyst formation occupied forty-five minutes. Of this time the withdrawal of the process *ps.* in Fig. 20 took about twenty minutes. During this interval rotation of the organism occurred.

Cross-infection of the Host.

The method of cross-infection of the larvae of *Chironomus* is probably a casual one, namely, by swallowing the voided cysts of *Amoebae* from infected larvae. I can state that I have seen larvae of *Chironomus* begin to feed on the vegetable tubes of other *Chironomi*, and that voided castings containing cysts occur in these tubes. Casual infection by the mouth is now known to be a very common method of transference of protozoal organisms from host to host, and it is probable that such is the case here.

General Remarks.

Many parasitic *Amoebae*, often styled *Entamoebae*, are known to occur in the digestive tracts of Vertebrates. Some *Amoebae* are also known from the alimentary tracts of Invertebrates, but at present these are fewer in number. In insects probably the best known parasitic *Amoeba* is that recorded by Bütschli (1878) from the cockroach, and named by him *Amoeba blattae*. Further researches have been conducted on this parasite in recent years (see Schubotz (1905) and others). An *Amoeba* (*A. sagittae* or *A. chaetognathi*) has, I believe, also been recorded by Grassi from the arrow-worm, *Sagitta*.

Miall and Hammond (1900) in their excellent account of the Harlequin Fly (*Chironomus*) do not refer to the parasitic *Amoeba* described in this paper, though they mention other parasites of *Chironomus*.

The exact species of *Chironomus* larvae investigated is difficult to determine. The commoner ones are *C. dorsalis* and *C. plumosus*. Probably the larvae of one of these species yielded the material for this research.

Summary.

Amoeba chironomi, nov. sp., is distributed through practically the entire length of the digestive tract of the larva of *Chironomus*.

The body of *A. chironomi* varies from 15μ to 18μ in length and from 10μ to 12μ in breadth. The single pseudopodium may reach 15μ in length; one pseudopodium only is usually present.

Ectoplasm and endoplasm are well differentiated. A nucleus and a contractile vacuole are present. Food vacuoles are rare. The contractile vacuole resembles an iris diaphragm, consisting of a series of

fine, curved, radiating canaliculi, opening into a central space. The excretory products are faintly reddish in colour. The presence of a contractile vacuole is uncommon in parasitic *Amoebae*.

The nucleus is poor in chromatin. A nucleolus is present.

A. chironomi is highly sensitive to the degree of concentration of the medium in which it lives. Very slight increase in density causes the organism to encyst.

Encystment occurs in the rectum of the host, and the cysts are voided with the faeces. The cysts are from 12μ to 20μ long and from 9μ broad. The process of encystment is rapid.

The method of cross-infection of the host is probably a "casual" one, viz. by the mouth.

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SOME PHENOMENA ASSOCIATED WITH PARASITISM.

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(Three Figures.)

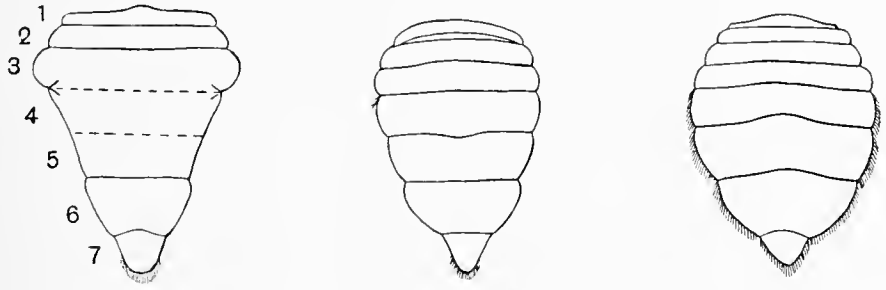
Introduction.

Observation on the behaviour of animals infected by parasites has generally been confined to direct effects, and such as mainly concern the invaded parts. It has of course often been shown that the constitution of the blood (at least with regard to its cellular elements) is altered in most kinds of parasitic affections even when a gut-parasite is in question. This indication of the indirect action of parasites leads to the consideration of cases like those below where the influence of the invading organism exerted through the medium of the blood results in definite changes of the reproductive system. Probably no other part of the organism responds in so marked a manner to the readjustment of the general metabolism which the parasite occasions, but there is still great possibility that changes of considerable interest may take place in other parts of the body.

I. *Changes in the Reproductive System of the Host.*

It is a common experience for an observer of marine nature to find, tucked under the abdomen of a crab, a yellow globular body which with its connected root system ramifying through the body of the host, has more the appearance of a pathological growth than a separate organism. It has however been known for more than seventy years that this curious parasite, to which the name *Sacculina* was given, is an aberrant cirripede. The curious nature of its life-history has

occasioned considerable controversy among the distinguished investigators of the problem, but extraordinary as are the proved changes which *Sacculina* undergoes in its development, yet another phenomenon results from its association with its host which is certainly of equal importance. It is of no small interest then, that while to Delage we owe the clear and convincing demonstration of the endoparasitic development of *Sacculina*, it is Alfred Giard, whose recent death we all deplore, who gave the first description of the effects that the same parasite causes in the reproductive system of the Crustacea it infects.



Abdomens of Shore Crab (*Carcinus*) infected by *Sacculina*.

Fig. 1.

In 1877 Fraisse was engaged on the study of the *Sacculina* parasitic on the spider crab (*Inachus*). In a paper published in that year he calls attention to the fact that the males of the infected species never harbour the parasite, and this he attributes to their narrow abdomen forming but an insecure lodgment for an attached organism. This instance of the sage discrimination of the infant *Sacculina* when, at the end of its free-swimming life, the time comes to choose a foster-parent, was refuted by Giard, who pointed out that the real reason of this apparent immunity was to be found in the tendency for the males attacked by *Sacculina* to exchange, at the moult subsequent to infection, their narrow abdomen for a broader one approaching the female type. He also remarked that the swollen chelae characteristic of the adult male spider crab are represented in the parasitised form only by the attenuated pincers usually associated with the female sex. For these reasons Giard considered it unnecessary to figure a crab of this kind, for "it would be indistinguishable from the classical figures of the female sex." Besides this well-marked effect in the infected male the French observer detected an equally definite assumption of male

characters by the female. Later research however does not support his conclusion.

In addition to describing the remarkable series of changes in the secondary sexual characters, Giard elucidated a second cardinal point, namely that the influence of the parasite on the genital glands of the hosts is such as to cause dwindling and subsequently entire disappearance. This induced sterility, in some Crustacea the invariable accompaniment of these associations with *Sacculina*, accounts for the name bestowed on this phenomena by its discoverer—"la castration parasitaire."

A long series of observations form the continuation of this early work and it is sufficient to say that the occurrence of changes as the result of parasitic infection have been demonstrated in most animal phyla either directly by Giard or others inspired by his researches. The cases of sterility accounted for by internal parasitism are now very numerous; but in few of these are the secondary sexual characters so well defined as to exhibit the corresponding changes in the external characters. The instances which most fully exhibit the nature of the phenomenon are all found in the Arthropoda where the possession of a chitinous exoskeleton allows of the expression of very definite sexual differences.

One case may well be quoted here. The singular modifications to which bees of the genus *Andrena* are subject, are traceable in every case to the fact that they harbour the larvae of the extraordinary insect *Stylops*. The phenomena of "stylopisation" have always been familiar to entomologists and have rendered the systematic study of these Hymenoptera exceedingly difficult. So distinctive are the changes which this insect parasite produces, that many species have been erroneously grounded on parasitised forms.

It must be pointed out that alterations of the reproductive system are nearly always due to parasites which exist in the body cavity and blood spaces of the host. This class might well be expected to exert a more powerful influence on the general metabolism of an infected organism than the intestinal parasites. Save for two exceptional cases described at the end of this paper we know of no cases of connection between the existence of large numbers of parasites in the gut and anomalies in the sexual organs. Before leaving the discussion of the scope of the phenomenon it may, too, be pointed out that no such effects have yet been observed in vertebrate animals. The extension of helminthological studies has resulted, for example, in the discovery

of toxin-secretion by parasites and its effect upon the blood of the host. This is an example of the important conclusions which are to be reached from investigation of parasitism in the Vertebrata, and it may be suggested that observation of the reproductive organs in acute cases of infection like filariasis and ankylostomiasis might prove of great interest[1]. It is perhaps fair to conclude that a marked change in the reproductive glands themselves would have been chronicled in clinical observations had it existed, but we may expect perhaps to find minor changes in the sexual economy or the secondary characters, traceable to the influence of a specific change wrought by the parasite.

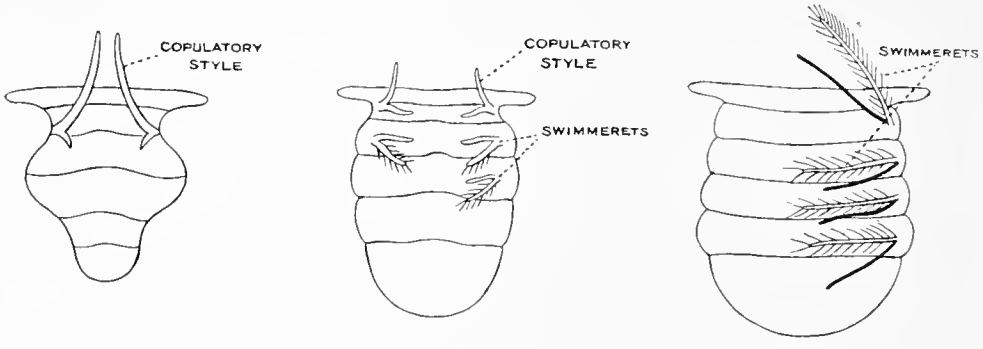
A preliminary notion of the nature and extent of the changes in the genital system due to parasitism has been given and it is now proposed to bring forward the evidence from later research which makes it possible to form a much clearer idea of the phenomenon.

Geoffrey Smith in 1906 published a very full account of the effect of the association of *Sacculina* with the spider crab (*Inachus*) embodying researches extending over three years. Before attempting to summarise this remarkable work, a little definite information about the life-history of the parasite and the sexual differences of the host must be given.

The larvae of *Sacculina* are free-swimming and are not transformed directly into the individual we know as the adult, but pass through a wholly internal existence in the body cavity of a crab, where they rapidly absorb nourishment from the blood-serum. A definite position in the abdomen of the host is attained, a closely ramifying system of absorptive roots is developed and the time approaches when the parasite becomes partly external. This change in the even course of its life can only take place through the agency of a moult. In the new cuticle which forms under the old carapace during moulting, modifications are found which concern the secondary sexual characters and these, it must of course be concluded, are due to the influence of the embryonic though maturing *Sacculina*. The infected crab never moults again while its parasite lives, indeed such a moult would be fatal to the parasite, and so while the gonads are subject to the constant action of the *Sacculina*, the external sex characters only preserve for us the early effects of the association. It is impossible certainly in the present case to observe whether the changes become

[1] Turner (1908) mentions the widespread belief in Mozambique that impotence and sterility accompany bilharziosis. As an interesting sidelight on the relations of parasite and host we may quote his statement of the frequent disappearance of the parasites at the onset of puberty.

progressively greater in degree. In a hermit crab in which very similar phenomena are evoked by the presence of another parasitic cirripede *Peltogaster*, moulting is not prevented and it is easily observed that even those individuals in whose secondary sex characters little or no modification could be traced (a condition which might be ascribed to the shortness of the period of parasitism previous to the last moult), never showed at subsequent moults the least tendency to increase the degree of modification with the prolongation of the period of infection. So many similarities exist between the two cases, that we are probably justified in assuming that the maximum alteration of which the individual host is capable is established at an early stage of parasitism in the spider crab as well as in the hermit crab.



Male Modified Male Female
(swimmerets of one side only shown)

Abdomen of Spider Crab (*Inachus*), under view.

Fig. 2.

The female spider crab possesses small flattened *chelae* or pincers, and can be also identified by the broad trough-shaped abdomen. The ventral surface of the thorax is much hollowed out so that between the thorax and the reflexed abdomen a spacious cavity exists where the eggs can securely run the course of their development. From the second, third, fourth and fifth abdominal segments spring pairs of long biramous appendages (*swimmerets*) the inner branches of which are richly provided with branching hairs, and it is to these last that the eggs are securely cemented. In the ways thus described the female is provided with such adaptations of structure as enable her to cope with the cares of motherhood, providing a safe attachment for the embryos and a brood-chamber for their protection. The male type of abdomen is much narrower and rather shorter and the thorax is not scooped out

so noticeably to receive it. There is nothing in the male to represent the egg-bearing swimmerets of the female: the first two abdominal segments only bear appendages, which are modified to form copulatory styles. The chelae are larger than in the female, the terminal joint having a swollen tumid appearance.

A description may now be given of the deviations from the normal found in infected crabs. The greatest number of the male spider crabs with *Sacculina* possess flat chelae of female type. In speaking of the chela as the organ which responds soonest to parasitic influence mention must be made of the fact that during the winter, which is the non-breeding season, many of the males, which are not, as far as can be observed, subject to the attack of any parasite, possess the flat female chela, a condition which is associated with a temporary suppression of the gonad. Both these phenomena involving chela reduction are the expression of a state of reproductive quiescence[1].

The other characters which are influenced by parasitism show no variation at any time from the typical form in the uninfected male. With regard to the shape of the abdomen more than half the infected crabs of male sex exhibit a condition intermediate between the narrow male type and the larger and broader organ of the other sex. Some undoubted males have a type of abdomen to be classed not as intermediate but wholly similar to the female.

The assumption of abdominal swimmerets is a characteristic only of the more highly modified male crabs. Thus only eleven out of one hundred and forty examined for this particular purpose by Smith showed their appearance. In these few cases great variation in the number of appendages occurred but some exhibited the complete series of four pairs, and both here and wherever the swimmerets were developed they were seen to differ from those of the mature female only in their rudimentary development.

There is of course a need of close observation in identifying the sex of these highly modified forms. All stages exist between the unaltered male and the individual which has assumed nearly all the external characters of the female. Such highly modified cases are in fact only to be distinguished at a glance from the female by the presence of recognisable

[1] The inference is that these "suppressed" males, after a moult shortly preceding the breeding season, appear with swollen chelae, by which time their testes have grown large and are in full reproductive activity. Though this case was not put to experimental proof there is no doubt that in *Cambarus*, a fresh-water crayfish, a form with well-marked secondary sex characters, passed by moulting into another form with slightly marked characters at the end of the breeding season (Faxon, 1885).

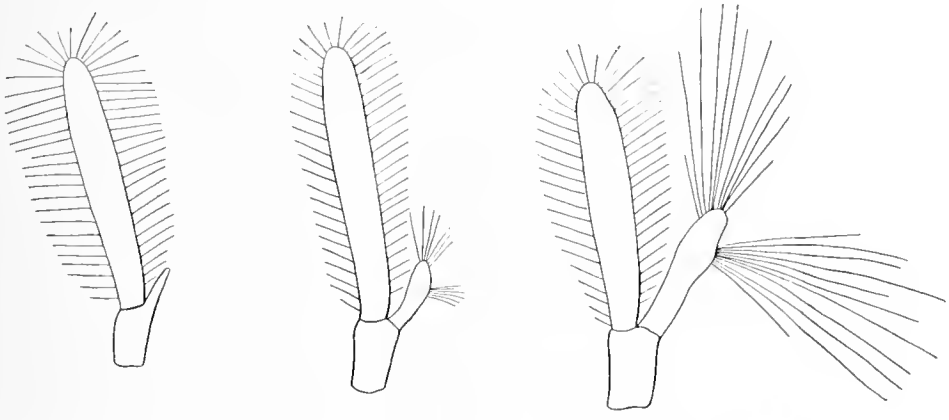
copulatory styles and by the possession of an abdomen which has not attained to the full female width. Nearly all such crabs show on dissection some vestige of genital gland which exhibits testicular structure.

A large number of infected spider crabs occurs, however, which cannot be assigned without careful thought to either sex. They have an abdomen of the exact female proportion, copulatory styles and abdominal swimmerets. Moreover the closest search in the vast majority of cases fails to distinguish any remnant of reproductive gland. But in two or three of the two hundred crabs of this class examined by Smith there were slender ducts and attenuated glands of an unmistakably male character. This piece of evidence is the first argument for regarding the whole of the class described above as modified males. When in addition, we consider that while individuals rigidly identified as males by the character of their gonads assume known female characters, the reverse change never occurs, so that for instance a crab which on dissection showed purely female glands has never been found to have developed even a rudimentary copulatory style or to assume a swollen chela, it seems justifiable to conclude that these crabs of debateable sex are really the last link in the chain of male modification.

In *Eupagurus,* a hermit crab, extreme modification also occurs as the result of parasitism by *Peltogaster,* another parasitic cirripede (Potts, 1906). This latter organism closely resembles *Sacculina* in its anatomy and life-history.

The difference between the sexes of *Eupagurus* is shown only in a couple of external characters, the position of the generative apertures (as in all Decapods) and the character of the abdominal appendages. The abdomen of the hermit crab is furnished on one side only with a few appendages, insignificant, but with definite functions. It is in the female that we see the full development of the appendage as a swimmeret with two equal branches, the inner one provided with long hairs affording a secure anchorage for countless eggs while the outer one is of equal size in both sexes, and in both by its paddle-movement maintains respiration currents in the shell. No use has been found for the outer branch in the male and so has become quite rudimentary, but the effect of the parasite *Peltogaster* is to stimulate the growth of this rudiment. There is of course great variability of response to this stimulus but those individuals which experience the maximum amount of change possess swimmerets exactly similar to those of a

mature female, even in the assumption of the curious bunches of barbed hairs which in this case can never bear eggs. As in the spider crabs so here, the female appeared incapable of the reverse change, and the large number of hermit crabs with typical female appendages and sealed genital apertures are undoubtedly to be regarded in part as modified males.



1. Unmodified Male 2. Modified Male 3. Fully modified Male or normal Female

Abdominal Appendages of Hermit Crab (*Eupagurus*).

Fig. 3.

A protest will conceivably be uttered against the attribution of a special sexual significance to the development of typical swimmerets in the male in both spider crabs and hermit crabs. It is of course well-known that in the larval stages of these Crustacea biramous abdominal appendages are found in both sexes to be subsequently reduced or lost in the male. Lest this, then, be deemed a happy opportunity for applying the term "reversion" to this phenomenon I hasten once more to point out that when the male develops biramous abdominal swimmerets they are of the type associated with female maturity, and that the specialised nature of their nursing-hairs cannot well be associated with ancestral conditions.

Both *Sacculina* and *Peltogaster* inflict sterility upon their host and apparently entire abortion of the gonad generally is the final consequence. On the external appearance of the parasite the eggs of the female shrink through absorption of their yolk, and the formation of spermatozoa is after a time suspended in the male. The testis of the spider crab dwindles and disappears without undergoing any

particular histological change; but in the hermit crab it is curious to note the presence of large cells with large nucleus and abundant protoplasm in sections of the testis. These instantly suggest ova in their appearance and call to mind the instances of the occurrence of such cytological elements as a normal experience in the testes of many animals. In sand-hoppers (*Orchestia*) to quote a well-known case (and there are many others in the Crustacea) spermatozoa are produced in the anterior part of the young testis while posteriorly the whole space is occupied by two or three large ova (*vide* Boulenger, 1908).

The particular interest of the phenomenon in this case is its association with a definite cause, that is, parasitism. We are also able to come to some conclusion as to the degree in which such a condition can be called true hermaphroditism. Some striking evidence is offered by spider crabs which were once infected by *Sacculina* but which have outlived their parasite and recovered from its influence. Such crabs occur in nature in fair frequency and the only reminder of their former condition is the chitinous ring on the abdomen which surrounded the peduncle of the parasite. After the death of the external part of the *Sacculina* the root system may continue to exist in the host and it is only when this has disintegrated and been absorbed that regeneration of the gonads becomes rapid, for the still living roots repress the development of the sexual organs as effectually as the living parasite. A few crabs however were found in which the gonads had again attained full size and maturity. One was a female with a well-developed ovary and four were males only slightly modified externally, with glands producing large quantities of spermatozoa. The remaining four cases were remarkable for the crabs showed with a complete external hermaphroditism the corresponding gonads. In all four animals the reproductive gland consisted of a male part with ripe spermatozoa, and a female division with large pigmented ova. The ducts were usually absent, but one individual possessed both vasa deferentia and oviducts. The sequel to these observations is given by the experimental evidence which Smith then obtained. It was attempted to destroy the parasite by removing the external part and the crabs so freed were kept under comfortable conditions for several months and the few survivors then killed. Regeneration had obviously occurred to a considerable extent, but the gonads were nearly always unisexual. In one individual alone, which was externally a hermaphrodite there was a gonad similar to those just described. In spite of the comparatively small number of cases with fully formed hermaphrodite glands we are not going too far

in definitely asserting a connection between their occurrence and parasitic influence, for bisexual gonads have to my knowledge never been met with in Decapod Crustacea under normal conditions[1]. But it thus appears that the curious condition in the hermit-crab is an incipient stage corresponding to the perfect hermaphroditism of the "recovered" spider crabs, and if the action of the parasite in absorbing surplus nutrition were withdrawn the young ova in the testis of the hermit crab would become large and pigmented like those in the spider crab[2].

These two cases have been described at some length as examples of *extreme modification*. In other Decapod Crustacea which are infected by the same parasite an effect is observable which is similar in kind but not in degree. The common shore crab of England (*Carcinus*) is commonly afflicted (if affliction it be) by *Sacculina*. Here again the male undergoes modification while the reverse change never occurs in the female. The narrow abdomen of the male is often exchanged at the moult after infection for one much broader but never attaining the full female width. One may look in vain however for any reduction of the copulatory styles or for the appearance of the smallest rudiments of swimmerets. The closure of the genital apertures nearly always follows parasitic attack in spider crab and hermit crab; but they never become blocked up in shore crabs with *Sacculina*. Yet the external change is apparently greater than that produced in the reproductive glands. Dissection in every parasitised male showed vasa deferentia of the characteristic milky white colour due to countless masses of spermatophores all packed with spermatozoa. The testes though reduced, then, always remain in reproductive activity. The parasites which infect spider crab and shore crab are practically identical and presumably exert a very similar stimulus, yet the results are markedly different. It is obviously the host which offers a different reaction in the two cases. In another crab (*Eriphia*) examined by Smith there was infection both by *Sacculina* and by a parasitic Isopod crustacean. Here the nature of the parasite governs the result, and crabs with *Sacculina* alone never showed the least trace of modification, while changes closely similar to those described above occurred in those which harboured the Isopod.

[1] Calman in the recently-appeared volume *Crustacea* of Ray Lankester's *Treatise on Zoology* refers to the unpublished observations of Wolleback on normal hermaphroditism in certain deep-water Decapoda.

[2] Experiments, like those of Smith's, planned to discover the fate of these ova in recovered hermit crabs were unfortunately inconclusive.

In discussing the general bearing of the whole phenomenon it need not be held that the diminution in size of the gonads and the changes in character of the sexual organs are directly connected. The first may be due to the mere drain of nutriment by the parasite, the second must have a more complex cause and is the expression of radical alterations in the metabolism of the host. Recent physiological experiment has gone far to establish the existence of *internal secretions* or *hormones* produced by many if not all organs (conspicuously the different parts of the reproductive system in Vertebrates) which maintain a most delicate interrelationship with the general metabolism. By some such secretion it has often been supposed that the gonads regulated and maintained the secondary sexual characters. It fits the facts better, here, to suppose, as do Geoffrey Smith and Heape, that the regulation of gonads and secondary sexual characters is due to a hormone circulating in the blood not regularly produced by the gonad. The action of the parasite in the male is to bring about an alteration in the constitution of the hormone, in fact there is substituted for the male hormone, partially or entirely, the very nearly related female hormone, or possibly an intermediate hermaphrodite secretion[1]. The production of female characters in a spider-crab not only in the absence of an ovary but in the presence of a testis shows the independence of secondary character and gonad, and it is to explain this that Smith's suggestion is put forward, while a very similar conception was arrived at even earlier by Heape (1901)[2] from consideration of the relations of the reproductive system in Vertebrata.

It is not easy to explain by what sort of mechanism the parasite is able to effect such a change in the metabolism of the host. Possibly a specific secretion is manufactured by the parasite which reacts with the sexual hormone, or, as Smith suggests, the latter is of such an unstable constitution that the mechanical interference of the parasite in the metabolism suffices to bring about its decomposition. The first hypothesis of specific secretion is capable of being put to the proof by a series of experiments involving administration of extracts of the parasite to a number of uninfected crabs and observation of the effects, on the lines so successfully established by Starling and Claypon in

[1] For this idea of a reproductive hormone differing in constitution according to sex and capable of alteration in the case of the male, Smith alone is responsible. It is significant that the chemical composition of the blood is known to vary widely in the vertebrata with difference of sex.

[2] Heape (1901), *Quart. Journ. Microsc. Sc.*, vol. XLIV., p. 66.

their classical experiments on the influence of extracts of foetus on the onset of lactation in sheep.

II. *Parasitism and Caste-Formation in Insects.*

In Grassi's description of the life-history of Termites (1893, 1896) we find a curious instance of "castration parasitaire" as an apparently normal condition in insect individuals. Termite colonies, as is well known, contain royal, worker and soldier adults all derived from the same kind of egg. Two kinds of larvae may be early distinguished, small-headed and large-headed respectively: while the latter produces workers and soldiers the former is the material from which the royal stock is replenished. The workers and soldiers are (in contrast to the Bee, whose workers are all female) of both sexes, but though the gonads are present they are of small size. By far the largest part of the abdominal cavity is occupied by a coecum of the intestine, whose excessive development appears to prevent the development of the gonads. The distension of the coecum is due to the presence of enormous numbers of parasitic protozoa. These organisms are absent in young larvae and in the fully developed sexual forms, but in those individuals, the soldiers and workers, whose relief from reproductive functions is apparently a necessary condition of their usefulness, the parasites occasion sterility. It is uncertain exactly how the repression of the gonads is brought about, but from the account it seems that the great size of the coecum leaves no room for the growth of the gonad so that the effect is a purely mechanical one. Though the cause is similar the mode of action differs greatly from that in the cases in the Crustacea discussed above. The root system of *Sacculina* and *Peltogaster* practically never comes into contact with the reproductive organs—the parasite must act on them indirectly.

This interesting piece of work shows not only the connection between parasitism and sterility but indicates how the termites control the appearance of sexually mature forms through diet-regulation. The principal food of the termites is wood, often rendered more nutritive by passage through the alimentary canal of another individual. But in certain cases, namely, the youngest larvae and those which are being transformed into sexual forms, the diet is the saliva of the workers, and since the recipients of this alone have no gut-parasites, Grassi concludes that the saliva is capable of destroying the protozoan fauna of the intestine. Should the royal pair come to an

untimely end certain larvae of the small-headed kind are fed on pure saliva, and the protozoa disappear. The growth of the sexual organs which then takes place is probably due to relief from the pressure of the coecum, though the absence of certain specific secretions of the parasites is conceivably a factor.

In a recent article on the "Polymorphism of Ants" Wheeler (1907) describes and discusses several cases of individuals occurring commonly in certain ant-colonies whose peculiar constitution he refers to "trophic disturbances induced by specific parasites." In the nests of the genus *Pheidole* very large and conspicuous ants attract the attention among the crowds of smaller workers and soldiers. They possess an enormously distended abdomen and attain to a length of 5 mm. (the workers do not exceed 3 mm.). Wheeler calculates that their volume is some twelve times that of the workers. Though not so markedly they also exceed the soldiers in length.

The great enlargement of the abdomen is in all cases seen to be due to the presence of a parasitic worm, determined as a species of the nematode *Mermis*. Wheeler concluded as the result of dissection that it was a parasite of the alimentary canal and did not lie in the body cavity. The fat-body and alimentary canal had alike disappeared and nearly all the available space was occupied by the distended crop. To these singular individuals Wheeler gave the name of *mermithergates*.

Infection must be effected while the future mermithergate is still a larva, but in pupal examples taken in association with adults the abdomen was not distended. The pupae were observed to hatch and at once the abdomen began to enlarge rapidly. The parasite is then of small size as this period begins but its speedy development is due to the supply of rich and abundant food to the newly hatched adult by the workers. Wheeler has graphically described the state of "chronic hunger" characteristic of the mermithergate and due to the growth of the parasite.

It may be pointed out that it is not only the digestive system which is hypertrophied but the "whole body exhibits excessive and uniform growth" with the exception of the head. Whether we consider the mermithergate as a modified worker or soldier, we must recognise the small head as a sign of incompetence to produce the powerful mandibles and attached muscles of the other forms, possibly because the parasite is able to appropriate the special head-forming substances. The huge head of the soldiers and some workers is a recently acquired character, and in some nests even scarcity of food appears to be responsible for

the production of small-headed workers only. Another feature of the mermithergate is the development of ancient characters which have been lost in the soldier, namely ocelli and larger eyes.

The mermithergate proves that parasitism may stimulate growth of the host, and in a footnote to his paper Wheeler quotes Montgomery as saying that crickets infected with *Mermis* are apt to be larger than normal individuals. We have shown above that in parasitised hermit crabs a similar tendency is found. Possibly a comparison with fowls may justly be made where the relief from the processes of reproduction which follow caponisation is responsible for the increased size of the capons.

Summary.

(1) The marked peculiarity in infection of Decapod Crustacea by certain parasites is the alteration of the sexual characters of the hosts (*castration parasitaire* of Giard). This has a twofold nature, viz.:—

(*a*) *Sterility. In both sexes* gonads dwindle and may entirely abort.

(*b*) *Assumption of characters of other sex. In the male* the external characters proper to the female are assumed in greater or less degree. The testis is transformed into a hermaphrodite gland, either while still infected by the parasite (incipient hermaphroditism of hermit crabs) or when freed from its influence (ripe ovotestis in spider crab). The reverse effects are not found *in the female.*

(2) This phenomenon occurs throughout the Invertebrata, but though sterility is a frequent consequence, the alteration of sex characters is never so definite as in the Crustacea. Analogous observations on parasitism in the Vertebrata have not been made.

(3) In the Insecta, parasitism appears to influence *caste production.* Grassi suggested that the sterility of soldiers and workers in Termites is due to protozoan parasites. In ants a giant caste is known (mermithergates) always harbouring a nematode worm.

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ON NEW SPECIES OF IXODIDAE, WITH A NOTE ON ABNORMALITIES OBSERVED IN TICKS.

By CECIL WARBURTON, F.Z.S., and
GEORGE H. F. NUTTALL, F.R.S.

(With 26 Text Figures.)

Since we published our last notes upon new species, a considerable number of specimens have reached us from various parts of the world, thanks to many gentlemen who have interested themselves in these parasites. We propose to group our descriptions of new species according to genera as we progress with the diagnoses we are preparing for the work on Ticks which we are publishing in conjunction with Messrs Cooper and Robinson. We have already described several new species of *Ixodes* and *Haemaphysalis* and in this paper shall confine our descriptions to species belonging to these genera. Although, more recently, we have received many specimens belonging to the genus *Ixodes*, we have, in the majority of instances, been able to refer them to established species, but sometimes with great difficulty owing to the meagre description given by many authors, and especially to the absence of figures. We consider that too much stress cannot be laid upon the necessity of illustrative figures accompanying authors' descriptions of new species for a glance at a figure frequently suffices as a means of rapidly identifying a species, and for this purpose the simplest text figures are usually amply sufficient.

In this paper we describe the following new species[1]:

		Origin.	Our catalogue No.
1.	*Ixodes fecialis* (♀)	Australia	650
2.	*Ixodes fecialis* var. *aegrifossus* (♀)	Australia	339
3.	*Haemaphysalis humerosa* (♂ ♀)	Australia	669
4.	„ *proxima* (♀)	Sumatra	540 c
5.	„ *hoodi* (♂ ♀)	Gambia	424
6.	„ *doenitzi* (♂ ♀)	Singapore	635
7.	„ *koningsbergeri* (♂ ♀)	Java	496 c
8.	„ *vidua* (♂)	Malaya	Collection of the London School of Tropical Medicine.
9.	„ *dentipalpis* (♂)	Malaya	
10.	„ *spiniceps* (♂)	Malaya	
11.	„ *bispinosa* var. *intermedia* (♀)	India	515 a and 327

Ixodes fecialis, n. sp.

Figs. 1 and 2.

Male unknown.

Female (gorged): body long, cylindrical, 8 × 4 mm., dark brown, with yellow scutum, capitulum and legs. *Scutum*[2] rather longer than broad (1·5 × 1·3 mm.), broadest near the posterior end; uniformly punctate except along the cervical grooves, which are otherwise faintly marked; the sides straight and diverging posteriorly; lateral grooves indicated by a prominent ridge of darker colour, sparsely punctate and parallel with the lateral border; scapulae blunt; emargination moderate. *Dorsum* minutely punctate, and thickly studded, especially in the posterior portion, with very short white hairs. *Capitulum*: base, a triangle, longer than broad, with wing-like lateral projections hollowed dorso-laterally to receive the palps; porose areas long-oval, rather near together, very close to the posterior border, which is somewhat sinuous; palps medium, flat, the second article twice as long as the third; hypostome truncate, with parallel sides, 2|2 equidistant files of stout teeth, 9 per file. *Venter*: vulva between coxae IV; spiracle rather small, transverse oval, somewhat pointed dorsally, far from coxae IV; anal groove

[1] The form of description is in accordance with others that we have published (Nuttall and Warburton, 1907, p. 392), unessential details being rigidly excluded.

[2] Recalling a conventional form of heraldic shield, hence *fecialis*.

long, ill-defined in front but apparently somewhat ogival; the sides nearly parallel. *Legs* yellow, slender. Coxae unarmed but with posterior border somewhat blade-like; coxa I with strong anterior projection embracing the *basis capituli* collar-wise, its posterior edge straight; coxae II—IV with posterior edge increasingly curved, coxa IV being subcircular. Tarsi tapering.

Described from one gorged ♀ from *Dasyurus geoffroyi*, Cranbrook, W. Australia, presented by the Hon. N. C. Rothschild. Collected March, 1900.

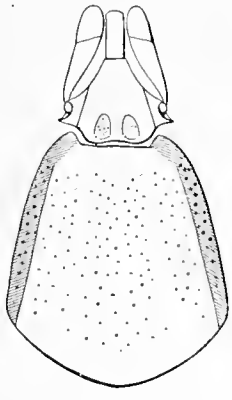

Fig. 1.

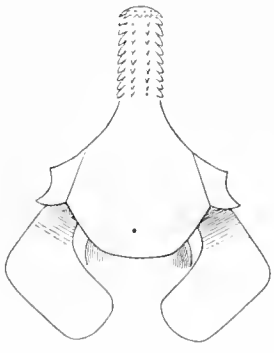

Fig. 2.

Fig. 1. *Ixodes fecialis* ♀. Capitulum and scutum, dorsal aspect. (C. W.)
Fig. 2. *Ixodes fecialis* ♀. Capitulum (palps omitted) and coxae I, ventral aspect. (C. W.)

Ixodes fecialis, var. **aegrifossus**, n. var.

Fig. 3.

Male unknown.

Female differs from the type in the following respects:

Scutum narrower (1·4 × 1 mm.), more pointed posteriorly, with punctations almost obsolete. *Capitulum*: base with the median triangular region distinctly marked off by ridges from the lateral regions; porose areas hardly visible (*aegrifossus*); hypostome ?

Described from three mutilated ♀s taken from an *Opossum*, Tamborine Mountain, Logan River, S. E. Queensland, April, 1907, and labelled "Scrub-ticks," sent by Professor R. T. Hewlett (London) who received them from the Bacteriological Institute, Brisbane.

N.B. The porose areas were at first believed to be absent, the median area of the *basis capituli* appearing to be uniformly finely granular in

texture. They were distinctly though faintly apparent, however, in a microscopic preparation.

These ticks possess a type of scutum, broad posteriorly, which appears to be somewhat characteristic of Australian forms. It is

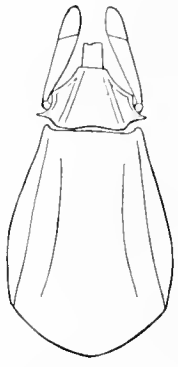

Fig. 3. *Ixodes fecialis*, var. *aegrifossus* ♀. Capitulum and scutum. (C. W.)

observable in *I. australiensis*, *I. ornithorhynchi* and *I. tasmani*, and the scutum of the nymph of *I. vestitus* is of this shape, though, in the adult female, it tends to become more circular. *I. tenuirostris* alone of extra-Australian species conforms to this type. The species here described most clearly resembles *I. vestitus* in general characteristics.

Haemaphysalis humerosa, n. sp.

Figs. 4 and 5.

Male livid yellow-brown, very long and narrow, 1·9 × ·9 mm. *Scutum* glossy, with very uneven surface due to more or less confluent punctations and ridges; cervical grooves long and nearly straight, slightly diverging posteriorly; lateral grooves long and very near the border, enclosing one festoon; festoons moderately long, the intervals curved. *Capitulum* ·3 mm. long, base rather long, with long sharp cornua proceeding rather from the dorsal surface. Hypostome with very small teeth (? 5|5). Palps long, especially article 2, which is about four times as long as article 3; its sharp external angle being much posterior to its insertion; no dorsal spur; a very small sharp spur under article 3. *Venter*: genital aperture between coxae II, covered by an elongate shield. Spiracles large, broader posteriorly, but without definite dorsal process. *Legs*: coxae very elongate and progressively increasing in size, all more or less pointed at the internal angle, the spur on coxa IV dark

and conical; coxa I protrudes beyond the scapular angle and rises to the level of the dorsal surface, giving a characteristic appearance (hence *humerosa*).

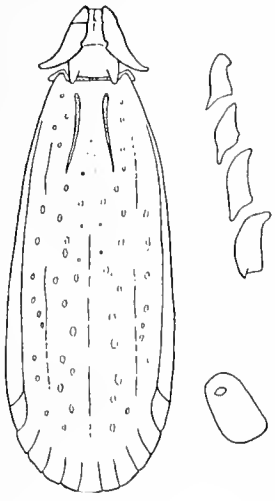

Fig. 4.

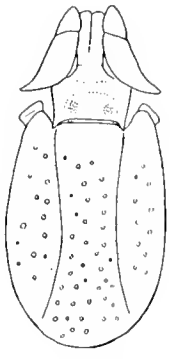

Fig. 5.

Fig. 4. *Haemaphysalis humerosa* ♂. Dorsum, coxae and spiracle.
Fig. 5. *Haemaphysalis humerosa* ♀. Capitulum and scutum. (C. W.)

Female the same colour as the male. *Scutum* oval, half as long again as broad (·9 × ·6 mm.); cervical grooves only slightly concave, reaching the posterior border. *Capitulum* ·4 mm. long, like that of ♂, but with article 2 rather less salient, and only about thrice as long as article 3. Hypostome with 5|5 very small teeth. Basis capituli ridged laterally and anteriorly, the sub-circular porose areas are very faintly indicated at the sides of the flat median area. *Legs* like those of ♂; tarsus IV medium, rather thick, tapering.

Described from 4 ♂ and 1 ♀ taken from *Perameles macrura*, Barrow Island, N. W. Australia. Presented by Hon. N. C. Rothschild.

Haemaphysalis proxima, n. sp.

Fig. 6.

Male unknown.

Female: body broad-oval, about 5 mm. long. *Scutum* 1·2 × 1·3 mm.; nearly circular, very finely and uniformly punctate; cervical grooves broad, shallow, extending for about two-thirds the length of the scutum; emargination rather deep. *Capitulum* ·7 mm. long; base rectangular with blunt cornua; porose areas long-oval, converging in front, far

apart; a median pit between them. Hypostome spatulate, with 4|4 sharp teeth. Palps much like those of *H. campanulata*; the outer contour sinuous; article 2 about twice as long as article 3, and sharply salient at its base. No dorsal spur; a long ventral spur under article 3.

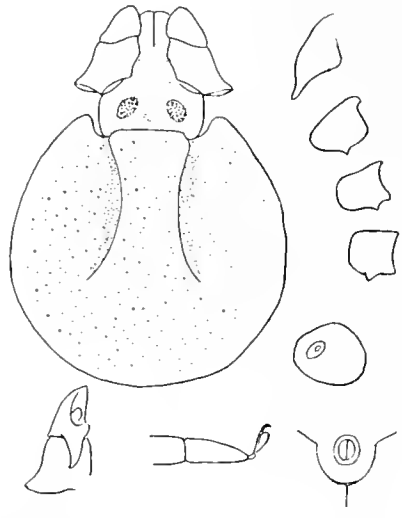

Fig. 6. *Haemaphysalis proxima* ♀. Capitulum and scutum, coxae, spiracle, palp in ventral aspect, tarsus IV, anal groove. (C. W.)

Venter: vulva small, level with the posterior border of coxa II. Spiracle large, sub-circular; anal groove forming a semi-circle behind the anus and diverging abruptly to join the genital grooves. *Legs*: coxa I prolonged to a moderate spur; coxae II and III rather blade-like internally and with a short conical spur near the middle of the posterior border; a rather stronger conical spur near the middle of the posterior border of coxa IV; tarsus IV rather thick, tapering gradually.

Described from a single ♀ taken from *Bos bubalus* in Sumatra, and sent by Dr J. C. Koningsberger.

This species is very closely allied to *H. campanulata*, from which it differs chiefly in the shape of the scutum and the tapering tarsi.

Haemaphysalis hoodi, n. sp.

Figs. 7 and 8.

Male: 1·8 × 1·3 mm. Body oval, rather broad, narrowing in front. *Scutum* much punctate with fairly large punctations; cervical grooves moderate, deep anteriorly, concave externally; lateral grooves well marked, of medium length, including the external festoon. *Capitulum*

rather short (·3 mm. long); base rectangular, punctate, with sharp cornua. Palps with article 2 very salient laterally at right angles to the axis; article 2 slightly longer than article 3; article 3 with a very small ventral process directed inwards. Hypostome well covered with equal teeth, 4|4, about 10 teeth per file. *Venter*: spiracle sub-rectangular, with blunt dorsal process. *Legs*: coxa I with blunt internal tooth; coxa II with a slight conical tooth near the internal angle; coxa III almost unarmed, a blunt spur at the inner angle of coxa IV; tarsus IV of medium length, abruptly narrowing at its tip.

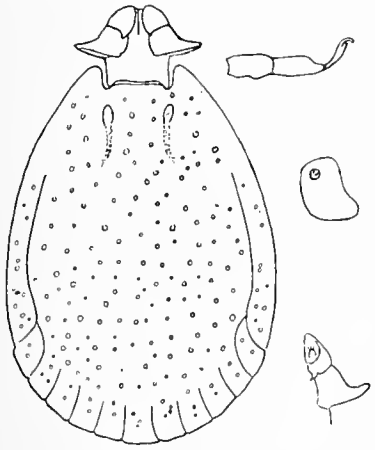

Fig. 7.

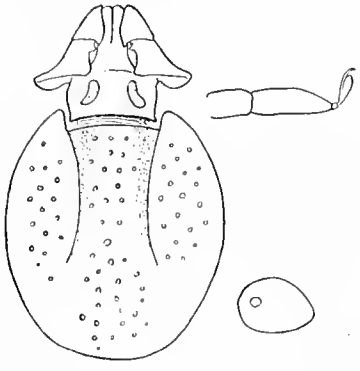

Fig. 8.

Fig. 7. *Haemaphysalis hoodi* ♂. Dorsum, tarsus IV, spiracle, palp in ventral aspect. (C. W.)

Fig. 8. *Haemaphysalis hoodi* ♀. Capitulum and scutum, tarsus IV, spiracle. (C. W.)

Female (unfed): yellow with darker border, well marked off by deep marginal grooves, which are nearly parallel; festoons square. When gorged, the scutum generally shows yellow on the dark body. *Scutum* oval, 1 × ·9 mm., truncated posteriorly, cervical grooves sub-parallel, extending about two-thirds its length; punctations fairly large and uniformly distributed. *Capitulum* ·4 mm. long; base sub-rectangular, with posterior border somewhat concave and cornua almost obsolete; porose areas rather reniform and situated anteriorly; well separated. Hypostome 4|4, like that of ♂. Palps longer than in ♂, especially article 2 which is not so abruptly salient laterally. *Venter*: spiracle rather large, egg-shaped, the pointed end dorsal; anal-groove semi-circular. *Legs*: like those of the ♂, but tarsus IV is longer and tapers less abruptly.

Described from numerous specimens of both sexes sent by Dr P. Hood, from Bathurst, Gambia, where it occurred on fowls in May, 1908.

Haemaphysalis doenitzi, n. sp.

Figs. 9 and 10.

Male 1·4 × ·8 mm., long-oval, narrower in front, glossy yellow-brown. *Scutum* finely punctate; cervical grooves nearly straight, converging posteriorly; lateral grooves long, curved, deep, including two festoons; festoons long, the dividing lines curved. *Capitulum* ·3 mm. long; base trapezoidal, broader in front, punctate, with short pointed cornua. Hypostome 4|4 (or 5|5?) with very small teeth. Palps long, articles 2 and 3 of about equal length, article 2 strongly salient at its base; article 3 without dorsal process, but with a very small ventral spine. *Venter*: spiracle ovate, bluntly pointed dorsally; anal groove rather ogival. *Legs*: coxae normal; tarsus IV of medium length, tapering rather abruptly.

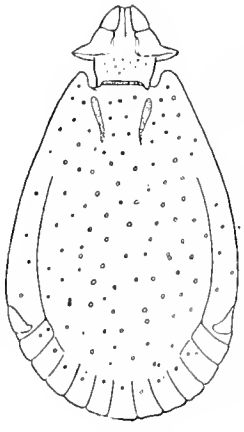

Fig. 9.

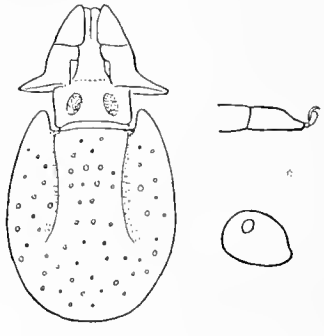

Fig. 10.

Fig. 9. *Haemaphysalis doenitzi* ♂. Dorsum. (C. W.)
Fig. 10. *Haemaphysalis doenitzi* ♀. Capitulum and scutum, tarsus IV, spiracle. (C. W.)

Female (gorged): broad-oval, 4 × 3·3 mm. *Scutum* oval, ·8 × ·6 mm., rather deeply emarginate, uniformly punctate with rather large punctations; cervical grooves sub-parallel, hardly visible for more than half the length of the scutum. *Capitulum* ·4 mm. long; base rectangular, with very slight cornua; porose areas long-oval, far apart. Palps like those of the ♂ but longer. Hypostome narrow, rather spatulate, with 4|4 files of very small teeth. *Dorsum* strongly and coarsely punctate. *Venter*: spiracle sub-circular, with blunt dorsal process; anal groove ogival. *Legs*: coxae and tarsus IV as in the ♂.

Described from 4 ♂ and 3 ♀ taken from a "water-hen" on St John's Island, Singapore, and received from Dr A. R. Wellington (1909), and named in honour of Geheimrath W. Dönitz (Berlin).

A very small species, closely allied to *H. hoodi*.

Haemaphysalis koningsbergeri, n. sp.

Figs. 11 and 12.

Male: 2·4 × 1·3 mm., elongate, broadest towards the posterior end. *Scutum* finely punctate; cervical grooves deep at first, then superficial, long, parallel; lateral grooves long but faint, except in the neighbourhood of the spiracle, including one festoon; festoons very long, with strongly curved intervals. *Capitulum* ·7 mm. long. Base trapezoidal, broadest in front, punctate, with short sharp cornua. Hypostome 4|4, about 8 teeth per file. Palps, article 2 very salient, much longer than article 3,

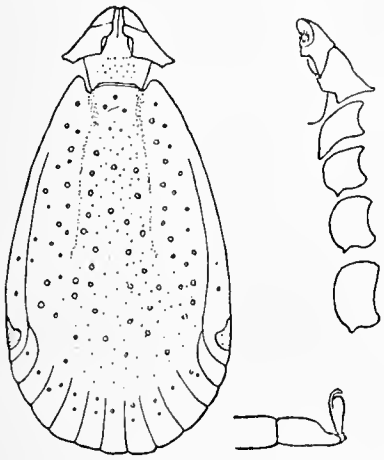

Fig. 11.

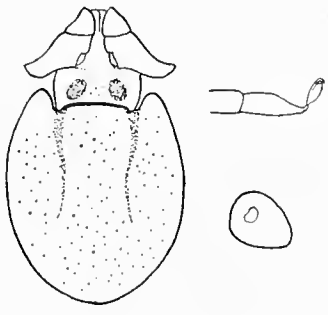

Fig. 12.

Fig. 11. *Haemaphysalis koningsbergeri* ♂. Dorsum, outline of palp and coxae, tarsus IV. (C. W.)

Fig. 12. *Haemaphysalis koningsbergeri* ♀. Capitulum and scutum, tarsus IV, spiracle. (C. W.)

the lateral contour of articles 2 and 3 nearly straight; no dorsal spur, but a rather strong sharp spur under article 3. *Venter*: spiracle oval, with blunt dorsal process; anal groove very slightly ogival. *Legs*: coxae increasing in length posteriorly, with the normal armature. Tarsus IV rather short and abruptly narrowing at the tip; pad large.

Female (gorged): broad-oval, 7 × 5 mm., punctate. *Scutum* 1·2×·9 mm., oval, deeply emarginate, rather pointed behind, finely punctate;

cervical grooves extending about two-thirds the length of the scutum, at first deep and convex outwardly, then superficial and curving outwards. *Capitulum*: base sub-rectangular, with rather convex sides and very slight cornua; porose areas long-oval, far apart, converging anteriorly. Hypostome 4|4, ten or eleven teeth per file. Palps much like those of ♂. *Venter*: spiracle ovate, the pointed end dorsal. *Legs*: coxae normal, tarsus IV longer and less abruptly tapering than in the ♂.

Described from 2 ♂ and 2 ♀ sent by Dr J. C. Koningsberger of the Zoological Museum at Buitenzorg, Java, in 1908. Its host was *Felis pardus*.

Haemaphysalis vidua, n. sp.

Fig. 13.

Male: 1·8 × 1·1 mm., yellow, oval, broadest in the middle. *Scutum* with very numerous shallow punctations; cervical grooves deep oval pits with shallow divergent posterior continuations; no lateral grooves. *Capitulum* short (·3 mm.); base rectangular, comparatively long, with short blunt cornua; hypostome with large crown followed by 4|4 rather scale-like teeth, few in number, about 30 in all; palps with articles 2 and 3 about equal in length, and with article 2 salient near its distal end, and forming a sinuous contour with article 3; a ventral retrograde spur from the outer border of article 3. *Venter*: spiracle oval, with slight dorsal prominence; anal groove ogival. *Legs* not very strong;

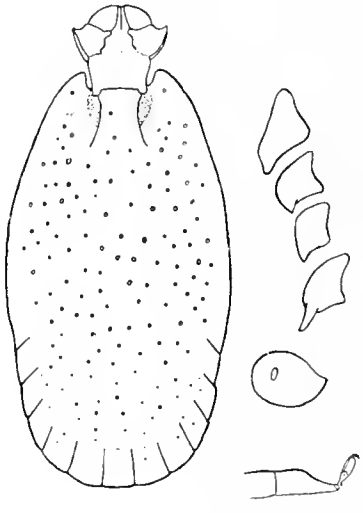

Fig. 13. *Haemaphysalis vidua* ♂. Dorsum, coxae, spiracle, tarsus IV. (C. W.)

coxae with a moderate spur at each postero-internal angle, strongest on coxa IV. Tarsi thick and abruptly tapering, almost humped.

Female unknown.

Described from a single specimen taken (in company with *H. koningsbergeri*) from *Paradoxurus* sp.? by Mr A. T. Stanton in the Malay States, and communicated to us by Lieut.-Colonel A. Alcock, C.I.E., F.R.S., March, 1909. (Collection of the London School of Tropical Medicine.)

Haemaphysalis dentipalpis, n. sp.

Fig. 14.

Male: body long-oval, 2·7 × 1·3 mm., broadest towards the hind end; curved, dull brownish yellow, the front legs, the capitulum and the anterior part of the scutum darker. *Scutum* showing conspicuous pseudo-

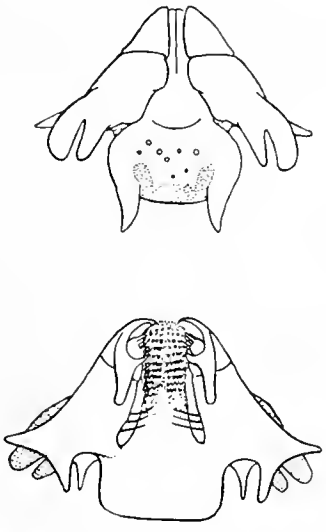

Fig. 14. *Haemaphysalis dentipalpis* ♂. Capitulum, dorsal and ventral aspects. (C. W.)

scutum, with numerous large, deep punctations; very fine punctations on the anterior portion; cervical grooves deep and convergent, then faint and divergent and visible almost to the posterior border of the pseudoscutum; lateral grooves short, beginning faintly at about half the body length, and including two festoons; festoons rather short and broad, with straight intervals. *Capitulum* ·6 mm. long; base broader anteriorly, with convex sides and rather concave anterior border; cornua strong, rather curved; hypostome long, parallel-sided, well covered with 7|7 very small equal teeth; palps long, conical, their dorsal surface concave;

article 2 much longer than 3 and with five retrograde projections, 2 dorsal and 3 ventral, best understood by reference to the figures; three simple dark hairs on the internal margin of article 2; a strong retrograde spine under article 3. *Venter*: spiracle very large, pear-shaped, with the narrower end dorsal; anal groove ogival. *Legs*: long, hairy, coxae like those of *H. koningsbergeri*, with a short conical spur at each postero-internal angle. Tarsi rather thick, abruptly tapering.

Female unknown.

Described from 4 ♂ taken (in company with *H. koningsbergeri*) from *Paradoxurus* sp.? by Mr A. T. Stanton in the Malay States, and communicated to us by Lieut.-Colonel A. Alcock, C.I.E., F.R.S. (Collection of the London School of Tropical Medicine.)

Haemaphysalis spiniceps, n. sp.

Fig. 15.

Male: body long-oval, 2·7 × 1·3 mm., broadest near the middle, glossy yellow-brown, darker on the sides and on the pseudoscutum. *Scutum* with large deep punctations anteriorly, some of them confluent and indicating by their distribution a pseudoscutum; punctations very small and numerous on the hinder portion; cervical grooves well marked, broad, nearly parallel; lateral grooves almost obsolete, only recognisable in the neighbourhood of the spiracles; festoons medium, punctate;

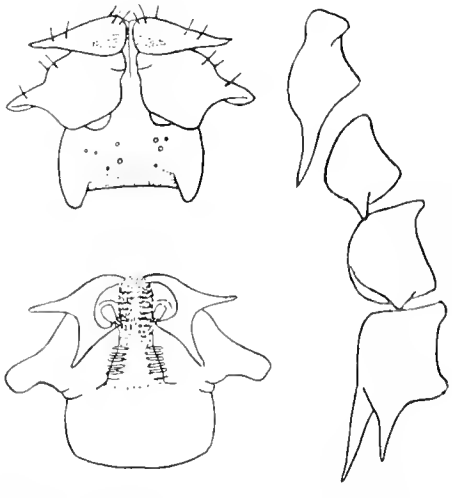

Fig. 15. *Haemaphysalis spiniceps* ♂. Capitulum, dorsal and ventral aspects, coxae. (C. W.)

the intervals dark and mostly straight. *Capitulum* ·6 mm. long; base broadly rectangular, with convex sides and strong cornua; slightly punctate; hypostome long, parallel-sided; well-covered with 5|5 small teeth; palps of remarkable form; article 2 strongly salient about the middle of its length and with five spatulate white hairs on its inner margin; article 3 with a strong, sharp, lateral spine and with a similar ventral retrograde spine directed downwards and outwards. *Venter*: spiracle very large and short comma-shaped; anal groove slightly ogival. *Legs* strong; coxa I prominent in front and with a strong spur at its postero-internal angle; coxa II with a blunt spur; a conical tooth about the middle of the posterior border of coxa III; two long spurs on coxa IV, the inner the longer. Tarsi tapering, pad long.

Female unknown.

Described from a single specimen collected by Mr H. C. Pratt in the Malay States (host not recorded), and communicated to us by Lieut.-Colonel A. Alcock, C.I.E., F.R.S., in March, 1909. (Collection of the London School of Tropical Medicine.)

Haemaphysalis bispinosa var. **intermedia**, n. var.

Fig. 16.

We received from Mr E. E. Green a specimen of *Haemaphysalis* taken by him from cattle at Matara, Ceylon, which, though very near *H. bispinosa*, seemed to differ from that species in important respects. Two

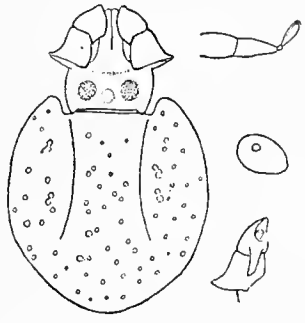

Fig. 16. *Haemaphysalis bispinosa* var. *intermedia* ♀. Capitulum and scutum, tarsus IV, spiracle, palp in ventral aspect. (C. W.)

other specimens (dry) taken from a cat at Agra, India, and sent by Dr E. H. Hankin in September, 1907, were evidently the same form. We were, therefore, led to revise our numerous specimens of *H. bispinosa*, and we found that two tubes, received from Lieut.-Colonel C. E. Nuthall, in

December, 1906, one containing ticks collected in the Bangalore and Mysore districts, and the other ticks from the Secunderabad and Hyderabad districts (hosts not mentioned) contained numerous females identical with the Matara specimen. Careful examination of these various ticks has convinced us that it is necessary to establish a new variety of *H. bispinosa*, especially as, in identifying that species, attention is naturally first directed to the dorsal spine on the third palpal article, which, in the variety, is almost obsolete.

H. bispinosa var. **intermedia** differs from the type as follows:—

Male unknown.

Female: scutum rather smaller (1 × ·7 mm.) and with sides somewhat straighter. *Capitulum*: base smaller, but in other respects similar. Hypostome 4|4, the toothed portion very short. Palps recalling those of *H. campanulata*, article 2 being abruptly salient, and the lateral contour of articles 2 and 3 being sinuous and unbroken. The dorsal spine on article 3 is nearly obsolete. Its situation is more internal, and it lies flush with the surface of the palp. The peculiarities of the capitulum give this variety a different facies from the type, in which article 3 of the palp is laterally salient, and the dorsal spine is raised conspicuously above the surface of the palp. It forms a link between the *bispinosa* group and the group of which *campanulata* is the type (hence *intermedia*).

Abnormalities observed in Ticks.

In the literature on ticks the occurrence of slight abnormalities has been occasionally noted. These abnormalities may affect a variety of chitinous structures. In *Argas persicus* slight asymmetry is not at all infrequent, as has been noted elsewhere[1]. In *Amblyomma hebraeum* var. *splendidum* (♂) we have observed the absence of the median festoon, and Oudemans' *A. scaevola* is abnormal in having the legs of one side underdeveloped. In *A. longirostrum* (♀), Cooper and Robinson (1908)[2] have noted that the spiracles may show asymmetry and in other Ixodidae the files of teeth on each half of the hypostome have been occasionally observed to be unequal in number. In a specimen of *Haemaphysalis bispinosa* received by us from India, the palps show a considerable

[1] Nuttall, Warburton, Cooper and Robinson (X. 1908), *Ticks, A Monograph of the Ixodoidea*, Part I, Argasidae, p. 13.

[2] Cooper and Robinson (15. II. 1908), On six new species of Ixodidae including a second species of the new genus *Rhipicentor* N. and W. *Proc. Cambr. Philos. Soc.*, XIV. pp. 457—470.

difference in size. The abnormalities which we have encountered as the result of examining a vast material are usually slight and infrequent. We have, however, come across a few specimens which appear to be worth noting. Thus, in two lots of *Haemaphysalis punctata*, taken from sheep at Littlebourne, Kent (March and April, 1905), and for which we are indebted to Mr W. F. Cooper, we discovered a number of gorged females which had a peculiar pear-shaped appearance owing to the expansion of the posterior portion of the body; the anterior portion, comprising the legs, being but slightly distended.

In Fig. 18 we depict an abnormality in a male *Hyalomma aegyptium*, measuring 3·3 mm. in length, exclusive of the capitulum. This specimen was found on the horse in Germiston, South Africa, in company with normal specimens which were sent to us by Dr B. Brock. For comparison, we include a figure (Fig. 17) of a normal *H. aegyptium*, drawn from a specimen in our collection.

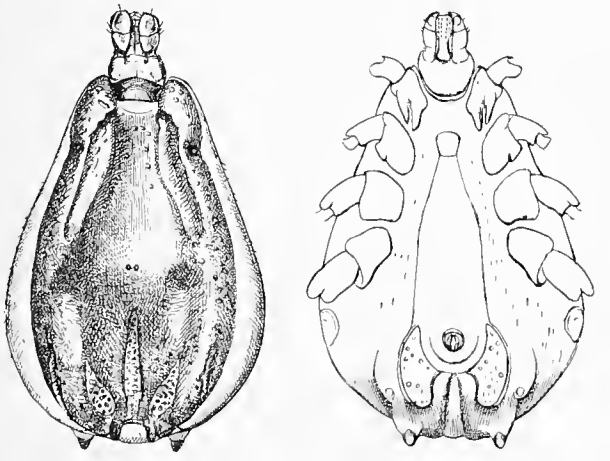

Fig. 17.

Fig. 18.

Fig. 17. *Hyalomma aegyptium* ♂. Dorsum and venter. Drawn from alcohol specimen by Mr E. Wilson under the author's guidance.

Fig. 18. *Hyalomma aegyptium* ♂. Posterior portion of venter showing abnormality. (C. W.)

Figs. 19 and 20 represent a normal ♂ specimen of *Boophilus decoloratus*. Fig. 21 gives the outline of a *B. australis* ♂ in which the adanal shields of one side are reduced to the merest rudiments, the corresponding side being much larger than normal. This specimen came from Borlesgama, Ceylon, where it was found on a cat in December, 1906. (Sent by Colonel B. Skinner, R.A.M.C.)

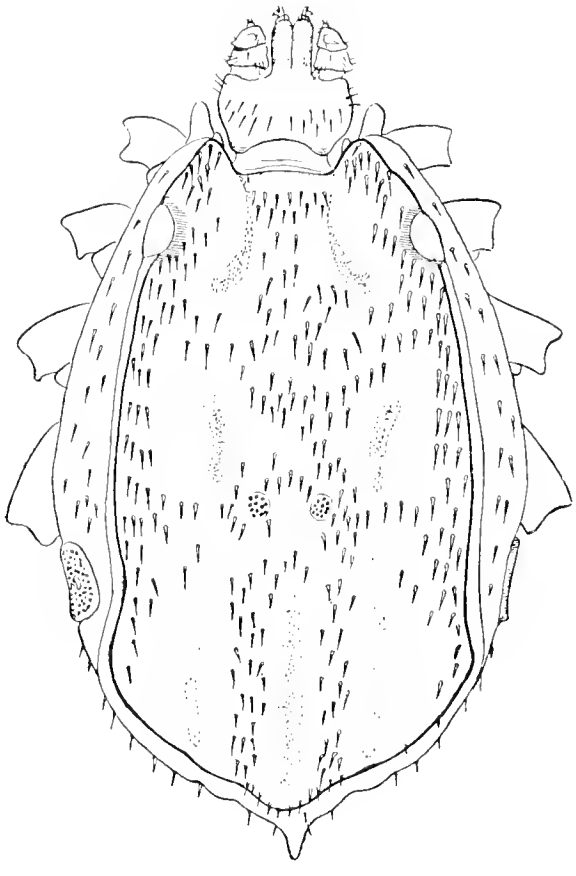

Fig. 19.

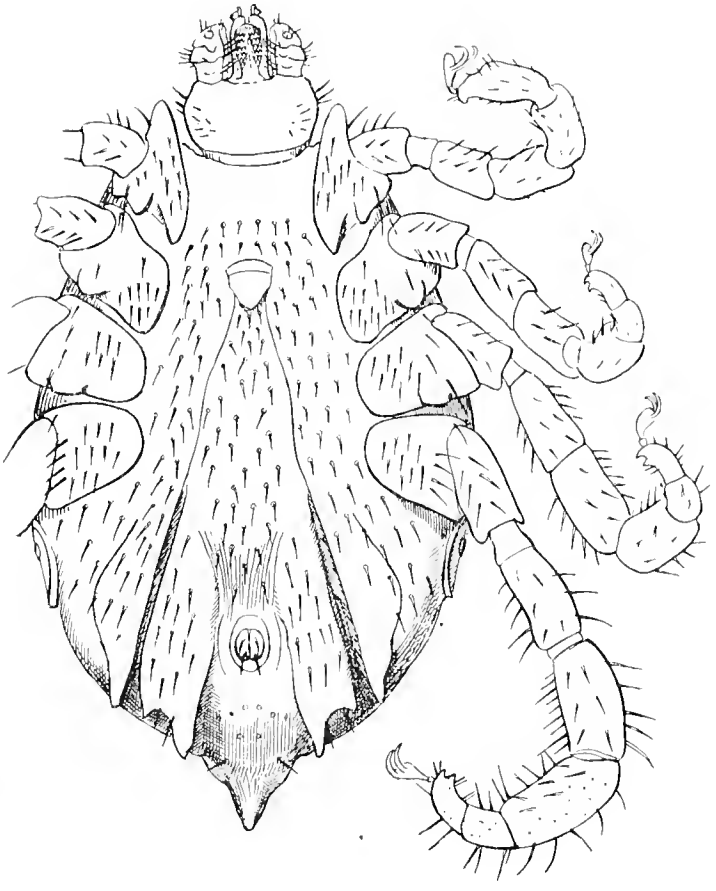

Fig. 20.

Figs. 19, 20. *Boophilus decoloratus* ♂. Raised in Cambridge from larvae received from S. Africa. ×30. The finely stippled areas on the dorsum indicate surface depressions or grooves. (G. H. F. N.)

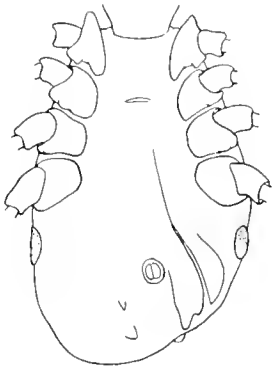

Fig. 21.

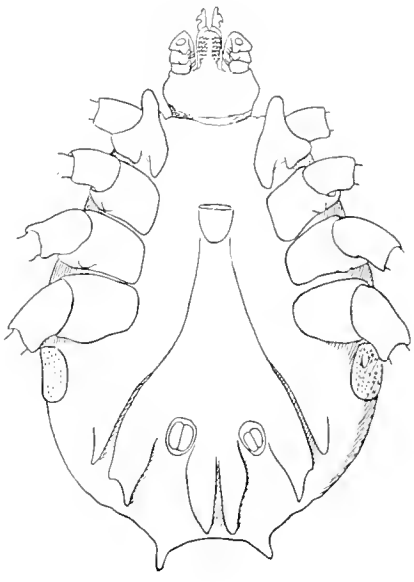

Fig. 22.

Fig. 21. *Boophilus australis* ♂. From Ceylon, showing abnormalities. ×21. (G. H. F. N.)
Fig. 22. *Boophilus decoloratus* ♂. Monstrosity. ×21. (G. H. F. N.)

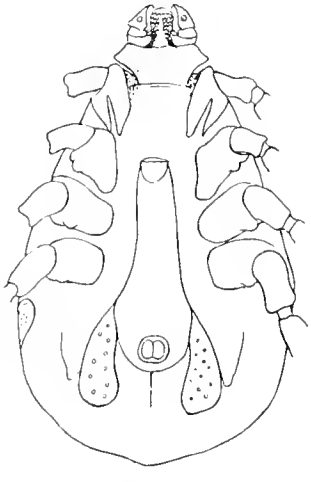

Fig. 23.

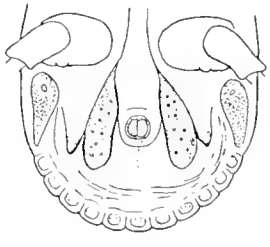

Fig. 24.

Figs. 23, 24. *Rhipicephalus sanguineus* ♂. Normal. Ventral aspect of fed (23) and unfed (24) specimens from India. (G. H. F. N.)

Fig. 22 illustrates a monstrosity, a *B. decoloratus* ♂ which was raised in Cambridge in July, 1907, from a batch of larvae sent to us from Cape Colony, by Mr C. P. Lounsbury. In this specimen there are two ani and two caudal spines, the median pair of adanal shields being slightly underdeveloped as compared to their mates on the outer sides

of the ani. This specimen was comprised in the same lot as the normal male depicted in Figs. 19 and 20.

In Figs. 25 and 26, the monstrosity, by duplication of the posterior parts of the body, is still more marked. We are indebted to Dr W. M. Graham (W. African Medical Service) for this remarkable specimen which he collected at Accra in June, 1908, off a dog. The specimen (*Rhipicephalus sanguineus* ♂, 2·8 mm. long) is more markedly teratological than the preceding specimen; the two adanal pairs of shields are completely developed and a single median circular spiracle is observable dorsally

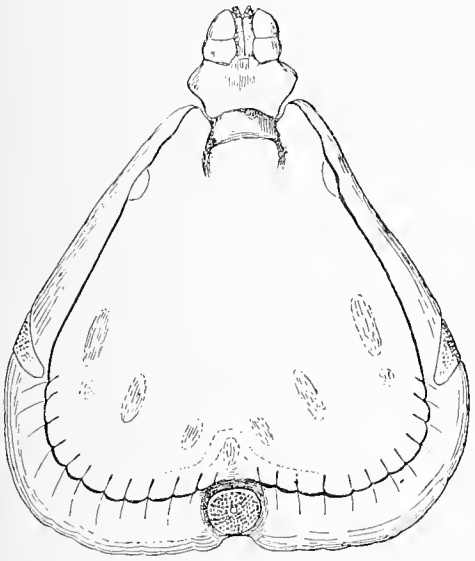

Fig. 25.

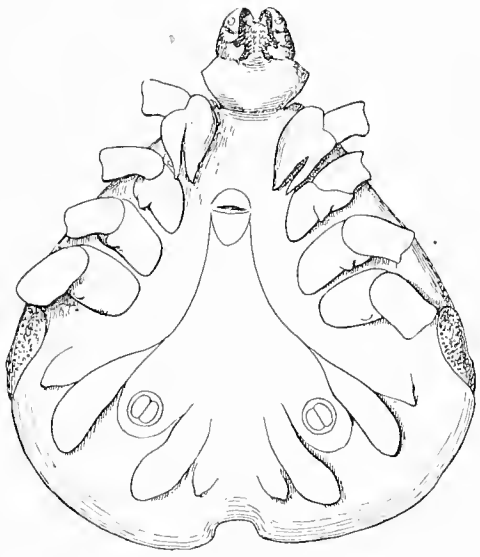

Fig. 26.

Figs. 25, 26. *Rhipicephalus sanguineus* ♂. Monstrosity. Length 2·8 mm. From W. Africa. (G. H. F. N.)

lying in a deep pit. The creature presents a fairly normal appearance anteriorly. There are 11 festoons on each side of the median spiracle; the dorsal grooves are normal externally but reduced medially owing to the fusion of the body in the median line. As in the specimen of *Boophilus* (Fig. 19) the legs appear normal.

To our knowledge, no similar instances of monstrosity have hitherto been recorded as occurring in Ixodoidea.

The expenses of these investigations are being partly defrayed by the aid of a grant from the Government Grants Committee of the Royal Society.

Note relating to the figures.

Figures 1—16 and 18 are from sketches made with the aid of a Zeiss binocular microscope (C. W.). Figure 17 was similarly drawn under our directions by Mr E. Wilson, the accuracy in detail being obtained by referring to drawings from mounted specimens. Figures 19—26 are reduced from drawings made with the aid of a camera lucida coupled with sketches from the opaque object (G. H. F. N.).

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THE STRUCTURE AND RELATIONSHIPS OF *GNATHOSTOMA SIAMENSE* (LEVINSEN).

By ROBERT T. LEIPER, M.B., F.Z.S.

Helminthologist to the London School of Tropical Medicine.

The natives of Siam are occasionally affected by subcutaneous tumours that have been found to be due to the presence of a small nematode worm named by Levinsen (1889) *Cheiracanthus siamensis*. Only one description of the species occurs in literature, and as this, the original one, was based upon a solitary immature female it is necessarily lacking in many details.

Recently a specimen was presented to the London School of Tropical Medicine by Dr A. F. G. Kerr, who had just returned from Siam. The parasite was mounted in Canada balsam and had become very opaque, as frequently happens to nematodes that have been prepared in this medium. By prolonged immersion in creosote the balsam was dissolved and the worm acquired considerable translucency. It was then evident that the specimen was a male, and therefore of very special value as it afforded material for a determination of certain important specific characters that had been hitherto unobtainable.

Description of the male worm.

The specimen measures 10·55 mm. in length, 0·6 mm. in breadth. The anterior half is quite straight and terminates in a distinctly globular swelling which is separated from the rest of the body by a neck-like constriction. The posterior half of the worm is involved in a ventral bending that developes into a complete spiral of one and a quarter turns before the tip of the tail is reached.

The globular swelling at the anterior end bears two large fleshy lips that guard the mouth. The cuticular covering of this portion is

provided with simple chitinous hooks arranged in eight transverse rows and having their sharp points directed backwards. These hooks measure 0·015 mm. in length and 0·005 mm. in greatest diameter.

The narrow neck measures only 0·3 mm. in diameter, but the body gradually increases in bulk until at a distance of 1 mm. from the anterior extremity it acquires a transverse measurement of 0·6 mm., which it maintains until within a millimetre of the tip of the tail.

For a distance of 4·5 mm. from the neck-like constriction the surface of the body is thickly beset with cuticular laminae that vary considerably in shape from before backwards, those most anteriorly attached have the free edge divided into three sharply tipped digitations, the succeeding plates eventually are found to be much narrower and to end in a single tip.

The posterior half of the body is entirely devoid of armature and the cuticle is quite smooth.

The alimentary canal.

The digestive tract extends along the whole length of the body as a broad tube which terminates in the cloaca within 0·15 mm. of the posterior extremity. The mouth is a simple opening devoid of vestibule and is guarded by two large fleshy lips 1·5 mm. broad and about 0·5 mm. high. Each lip shows a pair of sessile papillae and a median protrusion projecting between them. The lips give exit to the ducts of the paired labial glands that lie alongside the oesophagus in the perivisceral cavity.

The oesophagus is a highly muscular organ resembling that found in the Ascaridae. It measures in length 2·4 mm. and gradually increases in diameter from 0·13 mm. at the level of the neck to 0·45 mm. at its widest part just in front of the junction with the chyle intestine.

The chyle intestine has a fairly uniform diameter of 0·25 mm. The exact site of union with the rectum could not be ascertained.

Labial glands.

Four very remarkable organs, that from their appearance and connections seem to be excretory glands, surround the anterior half of the oesophagus. They are arranged symmetrically but are in no way attached to the oesophagus. Cylindrical in form and with rounded distal ends they hang in the perivisceral cavity attached only by their discharging

ducts to the body wall. These ducts pass into the circum-oral swelling and by the union of adjacent ducts each pair of glands discharges by a single common duct that perforates the adjacent lip. The glands measure 1 mm. in length by 0·1 mm. in breadth and show characteristic spiral markings upon their exterior.

Genitalia.

In the male four pairs of genital papillae surround the cloaca, two pairs lying pre-anally, two post-anally. They are large and nipple-like in shape, but decrease in size from before backwards. The cuticle overlying them seems to be detached from the body wall as a uniform fold, but there is no indication of the formation of anything resembling a bursa.

The two spicules are of similar design but differ markedly in length. Both are solid chitinous rods with a slight ventral curve ending in a blunted tip. They are unpigmented. The larger spicule measures 1·1 mm. in length, the smaller 0·4 mm. The specimen, like the previously recorded one, appears to be sexually immature. Owing to the indifferent fixation nothing can be made out of the disposition of the testicular tubule save that in the region of the cloaca there is a distinct muscular ejaculatory duct 1·5 mm. long.

The characters of the female worm, as given by Levinsen, are incorporated here for the sake of completeness.

"Corpus robustum, in dimidio modo anteriore spinulosum, lamellulis antice tridentatis, dente media dentibus lateralibus haud multo longiore. Pars capitiformis lata et brevis, seriebus octo spinarum instructa. Corpus in partem caudalem trilobatum desinet.

"Longit. 9 mm., latit. maxima 1 mm. Exemplar unicum (feminam) hujus vermis, a Deuntzer, medico in Bangkok inventi, examinari e tumore parvo pectorali (mammali?) feminae juvenis siamensis exhaustum. Exemplaria quinque vel sex ejusdem vermis e tumore similis alius hominis exhausta non conservata sunt."

Is Man the true host of the parasite?

The genus *Gnathostoma*, like its near relative *Tanqua*, is composed of species that are essentially intestinal forms. They live in the stomach of their host, giving rise to large fibrous thickenings at their site of attachment. *G. siamense* is the only species of the genus that

appears to depart from this rule and wander into the connective tissues. Moreover the forms examined by Levinsen and myself are both sexually immature. These facts taken together suggest that Man may not be the normal host for *Gnathostoma siamense* although the parasite is able to exist within the human body. Several instances might be quoted to show that where a parasite is able to survive in a closely related host it takes on a wandering habit.

The genus Gnathostoma.

The genus *Gnathostoma* comprises the following species:

1. *G. gracile* (Diesing) 1842, in *Sudis gigas.*
2. *G. hispidum* (Fedschenko) 1839, in *Sus scrofa.*
3. *G. horridum* (Leidy) 1856, in *Alligator misissipiensis.*
4. *G. paronai* Porta 1908, in *Mus rajah.*
5. *G. robustum* (Diesing) 1839, in *Felis catus.*
6. *G. siamense* (Levinsen) 1889, in *Homo sapiens.*
7. *G. sociale* (Leidy) 1858, in *Mustela vison.*
8. *G. spinigerum* Owen 1836, in *Felis tigris.*
9. *G. turgidum* Stossisch 1902, in *Didelphys azaree.*

The species *Gnathostoma shipleyi* belongs to the genus *Rictularia.* *G. robustum* of Diesing most probably is a synonym of *G. spinigerum*, Owen. *G. siamense* is very closely related to *G. spinigerum* and *G. paronai* and may eventually prove to be specifically identical with one or other of these two forms.

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ON THE SUPPOSED DEVELOPMENT OF *TRYPANOSOMA LEWISI* IN LICE AND FLEAS; AND THE OCCURRENCE OF *CRITHIDIA CTENOPHTHALMI* IN FLEAS.

By C. STRICKLAND, B.A.

(*From the Quick Laboratory, Cambridge.*)

According to Prowazek (1905) *Trypanosoma lewisi* undergoes sexual development in the rat louse, *Haematopinus spinulosus*. His description of the developmental process may be summarised as follows:

When *T. lewisi* enters the midgut of the louse, the nucleus undergoes changes, the chromosomes being reduced from 16 to 4. Then some of the trypanosomes become greatly attenuated, the reduced nucleus becomes band-like, and the cytoplasm stains differently. The attenuated forms are called the male gametes. They are seen to copulate with the female gametes by fusion at the non-flagellate end. The resulting zygote then develops into a trypanosome by the formation of a new kinetonucleus and locomotor apparatus.

Swingle (1907, p. 117), who examined fleas taken from rats harbouring *T. lewisi*, believed that he had observed a similar development to that described by Prowazek in the louse. He however did not describe any conjugation.

Woodcock (1909, pp. 196—273), who has lately reviewed the subject, believes that it has been "reasonably proved" that *T. lewisi*, like other trypanosomes, has a true alternative host. Although he has not apparently studied the subject at first hand, he concludes that the flea is the true alternative host of *T. lewisi*.

On the other hand MacNeal (1904, p. 520), working in America, could never trace any developmental changes of *T. lewisi* in lice (species ?), nor could Captain Patton, working in India, find any development of *T. lewisi* such as Prowazek has described in lice.

Nuttall (1908, p. 299) seriously doubts that any such development as described by Prowazek actually takes place. He inclines to the opinion that Prowazek was deceived by extraneous flagellates.

Patton and Strickland (1908, p. 323) "are forced to the conclusion that Prowazek has described part of the life cycle of a natural flagellate" and believe it has no connection with *T. lewisi.* They believe Prowazek has described in part a typical *Crithidia* natural to the louse.

Professor Nuttall (1908, pp. 296—301), whom I should here like to thank for his never-failing kindness and help, has recently confirmed the identity of the carriers of rat trypanosomiasis, and I have at the same time dissected a large number of rat-lice and fleas, with a view to following up any developmental phases which might occur in these insects.

The fleas were kindly identified for us by the Hon. N. C. Rothschild as *Ctenophthalmus* (*Typhlopsylla*) *agyrtes* Heller, with one or two exceptions hereafter named. The rats from which the insects were taken came from Cherryhinton, Cambs.

Since Prowazek traced the supposed development of *T. lewisi* in lice taken from rats in Berlin and Rovigno we tried to obtain a similar material to that with which he worked by importing wild rats (*Mus decumanus*) from Berlin, so that we might study the lice and trace the supposed development therein of *T. lewisi,* in view of the fact that we had signally failed to confirm Prowazek's results with material collected in this country.

The Author's Observations and Methods.

The insects (lice or fleas) were dissected in ·8% NaCl solution under a Zeiss binocular dissecting microscope, care being taken not to injure the intestine, which was isolated and examined in a fresh condition. At times special parts of the gut were divided off, teased in a *minute* drop of the saline solution, then dried rapidly in the air, fixed in absolute alcohol for 15 minutes and stained in Giemsa's stain. Coelomic fluid was obtained for examination by snipping off an appendage close to the body, whereupon a droplet of the fluid would exude from the wound.

The dissections and observations made may be grouped as follows: (*A*) Lice were examined from rats apparently not infected with *T. lewisi.* (*B*) Many lice were examined from rats more or less heavily infected with *T. lewisi* and observations were made upon the ingested trypanosomes in the alimentary tracts of the lice. (*C*) A few lice taken from rats infected with *T. lewisi* were dissected after fasting

for a variable period, and the state of the *T. lewisi* in their alimentary tracts noted. (*D*) Further observations were made on the state of digestion in lice which had fed on rats infected with *T. lewisi* and its relation to the presence or absence of *T. lewisi* in their alimentary tracts.

Similarly, fleas were examined from (*E*) infected and (*F*) apparently uninfected rats. The other organs of fleas and lice were also examined (*G*) for the possible presence of trypanosomes.

Finally notes are appended regarding (*H*) the occurrence of *Crithidia ctenophthalmi* in *Ctenophthalmus agyrtes.*

A. Examination of lice Haematopinus spinulosus *from apparently uninfected rats.*

Many lice were examined, which had been taken from rats whose blood had been carefully examined for *T. lewisi* with negative results. It is possible that some of the 42 rats from which the lice were taken may have harboured *T. lewisi* in such small numbers that they could not be detected microscopically. In not one louse however could any developmental forms of *T. lewisi* be found in the gut contents either in fresh or in stained preparations.

The following Table shews the number of lice examined:

Rat Ref. No.	No. of lice examined	Rat Ref. No.	No. of lice examined	Rat Ref. No.	No. of lice examined	Rat Ref. No.	No. of lice examined
3	1	22	10	43	5	91 B	17
4	3	26	2	44	5	68 B	5
5	11	27	10	46	8	92	
6	4	30	1	55	12	93 B	8
10	1	31	2	56	10	94	
11	1	33	5	60	2	IX†	8
12	1	34	6	61 B	13	—	—
13	1	35	8	63 B	16	—	—
14	1	38	5	66 B	16*	—	—
15	1	39	5	82 B	11	—	—
16	1	41	5	83 B	13	—	—
17	1	42	6	87 B	22	—	—
12		12		12		6	
	27		65		133		38

Total 42 rats, 263 lice.

The rats marked B were from Berlin. The sign * denotes that the examination of the lice took place 13 hours after their removal from the rats. † IX was a tame white rat.

Therefore 263 lice from 42 rats, apparently uninfected with *T. lewisi*, were examined, but no flagellate was found.

B. Examination of lice from rats infected with T. lewisi.

In a number of lice taken from rats more or less heavily infected with *T. lewisi*, it was found that active and typical *T. lewisi*, unchanged in every particular, persisted in various parts of the alimentary tract. In a number of cases no active trypanosomes were seen, they had disappeared entirely from the gut of the louse. The following Table shews the result of these observations, + indicating the presence of *T. lewisi*, − its absence.

Rat No.	No. of lice examined	Result	Rat No.	No. of lice examined	Result
2	2	−	52	1	−
18	2	+ in 2	54	4	+ in 1
29	6	−	57	15	+ in 8
32	4	+ in 1	64	3	+ in 3
36	1	−	65	13	+ in 10
40	3	−	88	2	+ in 2
47	8	−	89	15	+ in 11
48	2	+ in 2	90	23	+ in 13
8	28	+ in 5	8	76	+ in 48

Total 16 rats, 104 lice, 53+.

Therefore, 104 lice were examined from 16 rats, all infected with *T. lewisi*, and in 53 unchanged *T. lewisi* were found. No flagellates were seen in the other lice.

Where the trypanosomes still persisted in lice, they did not appear to affect special tracts of the alimentary canal, but they were never seen in either the oesophagus or rectum. Sometimes the trypanosomes were in the midgut only, sometimes in the hindgut only, in a few cases they occurred in the proximal parts of a Malpighian tube. In one stained preparation, a few *T. lewisi* were seen which appeared slightly changed and were probably undergoing involution, since they exhibited fragmentation of the nucleus and vacuolation of the cytoplasm. Rat 90, from which 23 lice were collected and examined, was suffering from a very chronic trypanosome infection.

C. Examination of starved lice.

A few lice were examined after fasting for a variable period. They were easily kept alive for 24 hours in a cool place, but they did not readily survive 48 hours fasting.

Rat No.	Hours of fast	No. of lice	Result
54	20	1	–
*69	18	1	+ very active
*70	24	1	+
*71	24	1	–
89	24	8	+ in 4
90	48	3	+ in 1
6	...	15	+ in 7

* signifies not recorded under section (B).

Thus 15 lice were examined after a fast of 18—48 hours, and in 7 *T. lewisi* still persisted, even in one case being very active after the louse had fasted 48 hours. No developmental changes were observed to occur in the trypanosomes.

D. The influence of digestion in lice on T. lewisi.

A number of observations were made on the state of the digestive process in the lice, as evidenced by the colour of the gut-contents, with a view to seeing if the persistency of the *T. lewisi* was in any way related to it. There did not appear to be any definite relation, for sometimes when the stomach of the louse contained bright red blood no trypanosomes could be found, whilst at other times, when the gut-contents shewed the last stages of digestion, trypanosomes were found.

The following Table gives the results of the observations made.

Rat No.	Louse No.	Colour of gut-contents	Result	Rat No.	Louse No.	Colour of gut-contents	Result
18	1	b. red	+	57	19	brown	–
	2	d. brown	+		20	b. red	+
40	3	b. red	–		21	b. red	–
	4	b. red	–		22	d. red	–
52	5	pale yellow	–		23	d. red	–
54	6	b. red	+	64	24	d. red	+
	7	d. red	–		25	brown-green	+
	8	red-brown	–		26	b. red	+
57	9	d. red	+	65	27	straw	+
	10	d. red	+		28	b. red	+
	11	b. red	+		29	b. red	+
	12	b. red	+		30	d. brown	–
	13	brown	+		31	d. brown	
	14	b. red	+		32	b. red	+
	15	b. red	+		33	straw	–
	16	red-brown	–		34	b. red	+
	17	red-brown	–		35	b. red	+
	18	brown	–		36	b. red	+

b. = bright. d. = dark.

Therefore 21 out of the 36 lice examined contained *T. lewisi* in their gut. The Table shews that the blood in the gut of the lice shewed all degrees of digestion. Whereas some of the lice whose intestine contained bright-red blood harboured no trypanosomes (3), the majority (14) contained *T. lewisi*. In lice, where the blood shewed evidence of having been more or less digested (19), only 7 contained trypanosomes. In these 7 lice, the digestion had proceeded to varying degrees, so that the gut-contents appeared dark red in three, dark brown in one, brown in one, greenish-brown in one, and straw-coloured in one.

E. Examination of fleas from rats infected with T. lewisi.

Taking now the fleas, *Ctenophthalmus agyrtes*, from infected rats, which were examined immediately after their removal from the rats, it was found that the trypanosomes could not be seen at all in the great majority examined, and that the examination of stained preparations of the gut-contents confirmed their absence.

The subjoined Table shews the result of these observations:

Rat No.	No. of fleas examined	Result
18	1	–
19	8	–
20	5	–
21	5	–
29	1	–
52	9	+ in 1*
53	3	–
54	2	+ in 1
55	6	–
56	3	–
84	1	–
123	1	–
12	45	

* Only one sluggishly moving trypanosome was detected.

Therefore 45 fleas from 12 rats infected with *T. lewisi* were examined, with the result that in all but two fleas *T. lewisi* had already disappeared.

F. Examination of fleas from uninfected rats.

Of the fleas from uninfected rats, not one shewed any trypanosomes in stained or fresh preparations.

Rat No.	No. of fleas	Result
20	10	–
30	1	–
42	5*	–
46	2	–
60	1†	–
92—94	11	–
124	1	–
9	31	

* signifies that one of these fleas was *Pulex irritans* and one *Ceratophyllus fasciatus.*
† *C. fasciatus.*

Therefore 31 fleas taken from 9 different and apparently uninfeeted rats shewed no *T. lewisi.*

G. The examination of organs, other than the alimentary tract, in lice and fleas.

In fleas which had fed on infeeted rats, other organs than the alimentary canal were examined in a few cases to see if any forms of *T. lewisi* could be encountered in these organs. Thus the generative organs, the salivary glands, and the fat body were examined. The coelomic fluid was also carefully examined. In none of these tissues could any traces of *T. lewisi* be discovered. I also dissected out the oesophagus of a few fleas, as this part of the alimentary tract was not removed when the midgut and the posterior parts of the intestine were examined. In the oesophagus, *T. lewisi* was never found, and the trypanosomes do not appear to migrate from the midgut forwards.

Similarly, in lice, which were collected from infected rats, the generative organs, the fat-body, the coelomic fluid and the oesophagus were prepared and examined, but no form of *T. lewisi* was encountered.

H. Notes on the occurrence of Crithidia ctenophthalmi.

In 8 fleas (*Ctenophthalmus agyrtes*), from 5 different rats infeeted with *T. lewisi*, I found *Crithidia ctenophthalmi.* This flagellate was almost invariably found massed in dense rosettes in the dilated upper part of the hindgut just below the openings of the Malpighian tubes, but tufts of them or single flagellates sometimes occurred as far down the gut as the rectum. Their movement was a lashing to and fro of the

body which remained perfectly rigid, while the flagellar end was attached to the gut-wall. Although I have never seen this parasite alive in the midgut of the flea, a group was discovered in a smear of the midgut which had been stained.

The rectal contents always contained a vast number of the barley-corn-like cysts of the *Crithidia*.

We have lately been successful in the Quick Laboratory in raising *Ctenophthalmus agyrtes* in large numbers, and it is hoped that we shall now be able to determine the complete life cycle of *Crithidia ctenophthalmi*. This flagellate was also found in one of the fleas from a rat uninfected with *T. lewisi*.

Summary.

1. The alimentary tracts of 104 lice (*Haematopinus spinulosus*), which had been taken from rats infected with *T. lewisi*, were examined. In 51 lice no trace of *T. lewisi* could be found. In 53 lice *T. lewisi*, unchanged in every particular, was found in various parts of the gut. The trypanosome was seen to be quite unchanged in stained preparations.

2. The alimentary tracts of 45 fleas (*Ctenophthalmus agyrtes*), which had fed on infected rats, were examined. The *T. lewisi* could not be found at all in 43 fleas. They disappeared very rapidly from the ingested blood and could not be found in stained preparations. In 2 fleas trypanosomes were found, but they did not differ in form from those seen in the blood of the rat.

3. 263 lice were examined from rats which were apparently uninfected with *T. lewisi*. No form of *T. lewisi* was discovered in fresh or stained preparations.

4. 31 fleas from uninfected rats were dissected and their alimentary tracts examined. No trace of *T. lewisi* was found.

5. 15 lice were examined 18—48 hours after their removal from an infected rat. *T. lewisi* was found in 7 of these, but they shewed no developmental changes.

6. Observations made upon 36 lice from infected rats shewed that at the earlier stages of digestion *T. lewisi* was more often found than at the later stages. In some lice however trypanosomes could not be found at the early stages of digestion, although they were present at an advanced stage of digestion.

7. The examination of other organs than the alimentary canal in fleas and lice did not shew any form of *T. lewisi*.

8. *Crithidia ctenophthalmi* was found in 9 *Ctenophthalmus agyrtes.*

9. About 370 lice and 75 fleas were examined but no evidence was obtained of developmental changes of *T. lewisi* in either fleas or lice.

Concluding remarks.

Professor Nuttall (1908) has proved that the transmission of rat trypanosomiasis can be easily brought about by fleas, and much less readily by lice. Consequently the flea is certainly the usual carrier of the trypanosome. The question arises whether this transmission depends upon these arthropods being true hosts of the trypanosomes. The observations recorded above afford no support to the view that the fleas and lice are true hosts of the parasite, nor that any sexual process of development of *T. lewisi* occurs in the arthropods.

Prowazek (1905) may have been misled by natural parasites of the louse, having no connection with *T. lewisi,* when he described the supposed sexual process of this trypanosome. If he saw a real stage in its development, we can say it must be rare, for we have never seen it.

On the other hand, it is not easy to understand the mechanism of infection. Jordan and Rothschild (1908) say that the proventriculus of the flea is designed to prevent regurgitation of stomach contents during feeding. Therefore it seems that infection by fleas is not effected by regurgitation of gut-contents. Possibly infection is effected by the inoculation of the minute drop of blood, which probably remains in the mouth-parts and around the opening of the salivary ducts in the pharynx of the flea.

With regard to the louse, it appears that the trypanosomes persist for a lengthy period in the gut. The louse being a constant feeder, it is almost certain that trypanosomes ingested at one meal would survive until the next. If then, as Prowazek (1905) asserts is the case, lice regurgitate the gut-contents, this seems a probable mode of infection.

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A CRITICAL REVIEW OF OUR PRESENT KNOWLEDGE OF THE HAEMOFLAGELLATES AND ALLIED FORMS.

By Captain W. S. PATTON, M.B. Edinb., I.M.S.

A large number of the trypanosomes and allied flagellates are now known to be the causal agents of some of the most fatal diseases of man and of the lower animals. The study of these parasites during the past fifteen years has truly made phenomenal strides, and at the present time it holds a position of commanding interest. Novy (1907) in a recent paper aptly states the reason for this active research when he says, "It is not merely because six or seven of these diseases have been recognised in domestic animals in Asia, Africa and South America. It is not wholly due to the discovery of a human form of the disease, but rather to the fact that the mastery by man of whole regions is called into question. The successful contest with malaria and yellow fever has now given place to the fight with trypanosomiasis and upon the issue of this struggle depends to a large extent the destiny of Equatorial Africa."

Of the numerous publications upon the Haemoflagellates the first to occupy our attention is one of recent date by Woodcock (Jan. 1909). Except for a few additions relating to recent work Woodcock's article is identical with a similar one which appeared in the *Quarterly Journal of Microscopical Science* for 1906. This in itself is disappointing for we understand that Dr Woodcock, as Mackinnon Student of the Royal Society, has been working for the last two years at the Haematozoa of birds and their relation to certain Haemoflagellates; yet very little convincing evidence is now brought forward to support Schaudinn's conclusions.

We propose reviewing in detail Woodcock's present article, which is divided into eight sections, a postscript, an appendix and a complete list of the known hosts of the trypanosomes and allied forms. In his

introductory remarks Woodcock states, that although the trypanosomes possess a common uniform type of organisation, they probably do not all belong to a single well-defined group of monophyletic origin, but are better regarded as an assemblage of forms which have sprung from quite different stocks. Their resemblance he regards as being brought about by the acquirement of similar adaptations to similar highly specialised modes of life. Later on we shall see that there is at present very little evidence in support of the many hypotheses on the origin of these parasites.

Haemoflagellates with two flagella, one directed anteriorly, and the other attached to the body of the parasite by an undulating membrane, Woodcock regards as being derived from a *Heteromastigine* ancestor. Those flagellates which only possess a single flagellum being derived from a *Monadine* ancestor; some of these however are to be looked upon as originating from a *Heteromastigine* form. All these flagellates possess a principal nucleus named by Woodcock the trophonucleus, and a smaller chromatic body, the blepharoplast, which Woodcock has named the kinetonucleus. The parasites divide by binary longitudinal fission or by multiple segmentation; very little however is known of their life cycles. The author gives a short account of the earlier work that has been done on the structure and life histories of the trypanosomes and pays a warm tribute to the great value of the Romanowsky stain and its many modifications. This is pleasing to read from the pen of a zoologist, as it was feared these valuable stains would have been condemned for the more recent wet methods now in vogue.

In the next section, Woodcock deals with the occurrence and transmission of trypanosomes, and notes that so far the majority have been found in mammals, birds and fishes, but a few have been described from amphibians, while among reptiles their occurrence has only been observed in one or two instances. In a recent paper Wenyon (1908) has described an interesting flagellate from the blood of a snake, *Erythrolamprus aesculapii*; this parasite is very suggestive of a true *Crithidia*. We are however at present unable to express a definite opinion on its nature.

Woodcock draws attention to the distinction between the true or natural hosts of trypanosomes and the strange or casual ones, and notes that when certain trypanosomes are injected into strange hosts, the latter readily succumb. This is naturally to be expected; it is difficult to understand any trypanosome not being pathogenic although the lesions it produces may not be gross. On more than one occasion we

have seen rats (*Mus decumanus*) succumb to an infection of *T. lewisi* and Jürgens (1902), Francis (1903) and others have made similar observations. The subject however is complicated by the production of active immunity against trypanosomiasis, and it is not therefore possible with our present limited state of knowledge to draw any final conclusions from the facts already ascertained.

Except in the case of *T. equiperdum*, trypanosomes are transmitted from one vertebrate host to another, through the intermediation of a blood-sucking invertebrate. Woodcock notes that the actual relations existing between any particular trypanosome and its transmitting invertebrate are at present uncertain, but that in most cases the invertebrate acts as a true host. According to Woodcock the investigations of Léger (1904), Brumpt (1906) and Keysselitz (1906) have made it clear that the trypanosomes of fishes undergo a definite evolutionary cycle in particular organs of leeches which have fed on the infected fish. The development however can only proceed to its fullest extent in a certain leech. For example, *T. granulosum* of the eel only undergoes its cycle of development in *Hemiclepsis marginata*; in other leeches, such as *Callobdella punctata*, *Hirudo troctina* and *Piscicola geometra*, the parasites only undergo partial evolutionary changes and then die off. This restriction, Woodcock says, distinctly points to the leech in question (*Hemiclepsis*) being the specific natural host of *T. granulosum*. It is true that Brumpt has succeeded in infecting healthy fish with trypanosomes by placing on them leeches already infected with the parasite; these experiments however by no means prove that this is the only method by which trypanosomes are transmitted from one fish to another. Thomson (1908), who has recently studied a trypanosome of gold fish, was unable to find a single leech on any of the fish from a particular pond, although a large number of fish were examined, the majority of which were infected. These observations clearly suggest that piscine trypanosomes may be transmitted by some method other than by the bites of leeches.

According to Schaudinn's (1904) researches *T. noctuae* undergoes its developmental cycle in *Culex pipiens* and there is a regular periodicity in the infectivity of the mosquito. Prowazek has also described the development of another vertebrate trypanosome *T. lewisi* in the rat louse *Haematopinus spinulosus*; and considers this insect acts as a true alternate host of *T. lewisi*. We would draw attention to Nuttall's (1908) recent transmission experiments with this parasite in which he clearly demonstrates that it is transmitted from rat to rat by means of *Cera-*

tophyllus fasciatus, Ctenophthalmus agyrtes and *Haematopinus spinulosus.* It is obvious from these experiments that neither of these blood-sucking insects are *true* alternate hosts of *T. lewisi,* more especially as it was not possible to demonstrate any developmental cycle of the rat trypanosome in their alimentary tracts.

It has been definitely established by Bruce, Nabarro and Greig (1903—1907) that tsetse flies can only transmit certain pathogenic trypanosomes of Africa up to forty-eight hours after feeding on an infected animal. Precisely similar results have been recorded by Minchin, Gray and Tulloch (1906), and in addition they ascertained by actual experiments that only the first animal upon which the infected fly fed became infected. These facts prove conclusively that these trypanosomes are conveyed by tsetse flies in a purely direct and mechanical way (see Appendix for Bruce's recent work). Minchin (1908) has however recently brought forward certain observations which suggest that *T. gambiense* undergoes some multiplicative changes in the alimentary tract of *G. palpalis.* After forty-eight hours the trypanosomes ingested by the fly are differentiated into two types which Minchin considers represent sexual forms; after ninety-six hours they however degenerate and disappear. It is impossible to say whether these changes represent part of the developmental cycle of *T. gambiense.* Koch (1906—07) also claims to have shown that *T. brucei* when ingested by *G. fusca* and *G. tachinoides* undergoes a true evolutionary cycle in the alimentary tracts of the flies, and lastly Stuhlmann (1907) has described what he considers to be the development of *T. brucei* in *G. fusca* bred in captivity. Woodcock, remarking on the above observation, says, that there is good reason to suppose that for a given pathogenic trypanosome there is a particular insect, which is a true alternate host, and that here, as among leeches, there are right and wrong hosts. He concludes this section by drawing attention to Minchin's (1908) recent hypothesis on the method of infection of trypanosomes. It will be remembered that this author's view is based on the discovery of the encystment of one of the flagellates of *G. palpalis* in the proctodaeum of the fly. As the transmission of trypanosomes is intimately connected with their life cycles we shall deal fully with this subject when considering the section devoted to the life cycles of these parasites.

In the section devoted to the habitat and effects of trypanosomes on their hosts, vertebrate and invertebrate, Woodcock first refers to Schaudinn's observations on *T. noctuae* in *Culex pipiens*; the extensive migration of the flagellates in the mosquito are quoted as an example

of the habitat of trypanosomes in an invertebrate host. We will criticise Schaudinn's observations in this connection further on. Prowazek's (1905) work on the development of *T. lewisi* in *Haematopinus spinulosus* is next described and the migrations of the so-called developmental forms of *T. lewisi* in the louse are said to agree in the main with Schaudinn's observations. Mammalian trypanosomes however have so far only been found in the alimentary tracts of tsetse flies; but Koch believes that the flagellates he found in the salivary glands of certain *Glossinae* are identical with *T. gambiense*. This is however by no means proved.

Woodcock next refers to Brumpt's (1906) work on the development of piscine trypanosomes in leeches, and notes that they also are restricted to the alimentary tracts of these invertebrates; in a footnote Brumpt's (1907) later observations on the hereditary transmission of certain leech trypanosomes are mentioned. According to Brumpt three kinds of behaviour of trypanosomes in leeches can be distinguished. (*a*) The parasites develop solely in the stomach of the leech and never pass into the intestine or sheath of the proboscis; this is the method of development of *T. remaki*. (*b*) In the case of *T. granulosum* the development begins in the stomach of the leech and is continued in the intestine, where the parasites may remain for a long time; they then pass back again to the stomach and later to the proboscis sheath. (*c*) This method of development goes on in the stomach, the parasites, *T. danilewskyi*, in time finding their way back to the sheath of the proboscis. *T. raiae* and *T. scylli*, which, according to Brumpt, develop in *Pontobdella*, undergo their evolution in the stomach and intestine of the leech, but Brumpt was unable to ascertain how they passed back again into the fish. Miss Robertson (1907) has recently described what she considers to be the development of *T. raiae* in *Pontobdella muricata*. We shall criticise these views regarding developmental cycles of piscine trypanosomes in the section dealing with the life histories of these parasites.

With regard to the effect of trypanosomes on vertebrate hosts, Woodcock gives a short *resumé* of the train of events in an infection with one of these parasites. We note however that Salvin-Moore and Breinl's (1907—08) recent work on *T. gambiense*, *T. equiperdum* and *T. lewisi* is only just referred to. We consider this work should have been described in detail. Woodcock draws attention to the great variation in the size and shape exhibited by many trypanosomes and states that in some instances at any rate sexual differentiation is

expressed by more or less pronounced differences in appearance. This is a very loose statement and should certainly be qualified, anyone reading it for the first time might be led to believe that the sexual types of trypanosomes are well established. As far as we can gather, sexual differentiation among these parasites is purely arbitrary, and there is not the slightest proof to show that certain short forms with coarsely granular cytoplasm are female parasites, while the more slender trypanosomes with few granules are males. That these forms represent true sexual differentiation and that the parasites conjugate to produce zygotes has yet to be proved (see Doflein, 1909).

We think Woodcock wisely refers to the two ends of a trypanosome as the flagellate and non-flagellate, we however do not doubt that the flagellate end is the anterior; it certainly is in *Herpetomonas* and *Crithidia*. Two remarkable figures of *T. lewisi* from the blood of a recently infected rat are given; their posterior ends are drawn out to a great length tapering to a point. Woodcock states that in such forms the flagellum is very short; we must admit we have not seen this stage of *T. lewisi*. About the fifth to the ninth day after a rat is inoculated with this trypanosome, young developing parasites are usually found in large numbers in its peripheral blood, and we have frequently noted that they may be readily distorted in making a blood film, particularly if it is fixed by the wet method.

In discussing the minute structure of trypanosomes Woodcock again appeals to Schaudinn's work and reproduces his figures of the development of an indifferent trypanosome (*T. noctuae*) from a halteridium ookinete. Salvin-Moore and Breinl (1907) have recently questioned the correctness of Schaudinn's interpretation of the appearances seen during the division of the nucleus of a trypanosome, claiming that the chromosomes are produced by the fixing and staining methods employed and that they are probably artefacts. If, as we believe, most of Schaudinn's figures represent changes in the development of certain insect flagellates, we have no doubt that their nuclei contain definite chromatic bodies, but whether these are true chromosomes is at present in our opinion of little importance.

Woodcock states there is no reason to doubt that the blepharoplast of a trypanosome is not merely an extra-nuclear centrosome, but a true nucleus homologous with and equivalent to the principal nucleus, and that there are distinct centrosomes connected with both blepharoplast and nucleus. The former he considers has an extra-nuclear centrosome at the base of the flagellum, and the latter an intra-nuclear

one. Salvin-Moore and Breinl, on the contrary, consider the blepharoplast is an extra-nuclear centrosome; Woodcock however considers that this view is not supported by the evidence furnished by the trypanoplasmes.

In the present state of our knowledge we think it would be of very little use to discuss the pros and cons of these disputes; we consider however that it is by no means proved that the trypanoplasmes afford any evidence on the structure of the trypanosomes. All the evidence so far collected (see p. 124) clearly suggests that true trypanosomes have originated from crithidia-like ancestors, and we believe that the study of these simple insect parasites will be a valuable guide to the study of the structure and origin of the trypanosomes. Quite recently the writer found a new flagellate in the Malpighian tubes of *Musca domestica*; this parasite is even more primitive in its morphology than any flagellate yet described, as its flagellum is entirely wanting and appears to be replaced by a simple elongation of its ectoplasm. It is hoped that the study of this parasite will throw some light on the nature of the flagellum and its connection with the blepharoplast; at present we think it is premature to theorize on the nature of these chromatic structures.

In the section devoted to biological considerations Woodcock remarks on the various movements exhibited by trypanosomes and notes there is a great difference of opinion as to whether the flagellum or the undulating membrane play the principal part in the movements of trypanosomes. It has always appeared to us that the flagellum is the chief factor in the movement of these parasites, and this is very evident when living Herpetomonads are studied. We have seen many which travel quite as fast as *T. lewisi.*

With regard to the peculiar phenomenon known as agglomeration, Woodcock states that, in the case of vertebrate trypanosomes, the parasites collect together by their aflagellar ends, forming a multiple union or rosette. In *Trypanosoma noctuae* according to Schaudinn the parasites agglomerate by their flagellar ends. A great deal of confusion is here introduced by the use of the term "rosette"; by this term we understand a group of parasites which have resulted from the multiple segmentation of a single cell. In cultures of *T. lewisi* (figured by Laveran and Mesnil, 1904) and in *Herpetomonas culices* (figured by Patton, 1907) the flagella are directed centrally; it should be noted these parasites are not *adult* flagellates. In the multiplication rosette of *T. lewisi* from the blood of a recently infected rat the flagella are directed

externally. In the case of the rosette of *Crithidia gerridis* the flagella are similarly directed externally, while in the agglomeration of the adult flagellates the flagella are directed centrally. The explanation of the position of the flagella in a true multiplication rosette is always readily ascertained by studying the method of multiple segmentation. In our opinion the agglomerated cluster of male forms of *T. noctuae* from the intestine of *Culex pipiens* as figured by Schaudinn represents a true Herpetomonad rosette. We think it is of some importance for a beginner to have a clear conception of the difference between a true multiplication rosette of a *Trypanosoma, Herpetomonas* and *Crithidia* and a mere agglomeration of adult flagellates, which is quite a distinct process.

It is not possible to say why adult vertebrate trypanosomes collect together (see p. 118) by their aflagellar ends under certain conditions. We have certainly not seen insect flagellates collect together by their aflagellar ends in the alimentary tracts of their hosts.

Woodcock considers the abnormal and involution forms of trypanosomes are produced as a result of chromatolysis, vacuolisation and change of form. In the light of Salvin-Moore and Breinl's recent work these round or oval bodies represent an important phase in the life cycle of a trypanosome; if this proves to be the case it opens up an entirely new field of research.

We now propose dealing with the most important section of Woodcock's article, the life cycles of trypanosomes, and we have pointed out above that it is intimately connected with the transmission of these parasites from one host to the other. When Woodcock wrote his first article on the Haemoflagellates Schaudinn's observations on the life cycle of *T. noctuae* appeared to be amply corroborated by the brothers Sergent (1905), Billet (1904), Brumpt (1904), Léger (1904) and Rogers (1904); we do not see however what connection the parasite of Kala-Azar has with Schaudinn's work and how Rogers' discovery of its flagellate stage has corroborated it. Woodcock evidently still thinks this parasite is a *Piroplasma* in spite of recent researches.

Since the publication of the paper by Novy and MacNeal (1905) describing valuable work on the trypanosomes of birds, Schaudinn's work has been largely discredited. Influenced still further by their researches on mosquito flagellates, Novy and his collaborators have expressed the view that the Haemoflagellates and Haemosporidia are quite distinct, and that the trypanosomes of vertebrates do not undergo any developmental cycle in insect hosts. In spite of what Woodcock says to the

contrary, that recent researches are in favour of vertebrate trypanosomes having specific alternate invertebrate hosts, we hope to show that the views of the American observers hold good at the present time. Woodcock then proceeds to describe the researches which support his contention.

According to Léger (1904), *Trypanoplasma varium*, in the blood of the loach, may be differentiated into ordinary (indifferent) forms and large, more granular (probably female) parasites. When these parasites were ingested by *Hemiclepsis* sp.? the indifferent forms degenerated and died out, the female parasites however became more massive and exhibited nuclear changes which Léger thinks suggest a sexual process. After some days the intestine of the leech contained numerous little narrow trypanoplasmes, some of which were filiform (male), while others had a kind of beak which made them resemble trypanosomes. These observations were published in a preliminary note without figures so that we are unable to refer to the actual appearances of the parasites. We know of no subsequent work confirming Léger's observations. In this preliminary note we are not told how *Trypanoplasma varium* developed into the male forms he describes from the leech; where the evolution of the one begins and the other ends is not at all clear. If these changes take place naturally in the leech it is surely possible to trace all the stages from the unchanged female forms up to the formation of the narrow trypanoplasmes and the parasites with beak-like processes; if not there must be some other explanation for the origin of these forms. We can find no reference to the possibility of *Hemiclepsis* being infected with a natural flagellate. We are therefore unable to accept this developmental cycle of *Trypanoplasma varium* as proved.

Woodcock next describes Léger's (1904) observations on *Trypanosoma barbatulae* in another leech, *Piscicola*. Léger, it will be remembered, draws particular attention to the formation of male, female and neutral forms of this parasite and says they correspond exactly with Schaudinn's three types. Woodcock states that these types are sharply differentiated in the invertebrate host and less so in the vertebrate. Here again we fail to see what connection these so-called male, female and indifferent forms in the leech have to do with *T. barbatulae*. The existence of these forms is certainly not proved in Léger's paper, a natural flagellate of *Piscicola* is not referred to, nor is it shown how the trypanosome gets back again to the fish. In his original article on the Haemoflagellates Woodcock says, "the above-stated facts however hardly leave room for doubt that both these piscine trypanosomes have a true alternating

Hirudinean host." We consider there is a great deal of room for doubt.

Woodcock next deals with Brumpt's (1906) observations on the development of *T. granulosum* in *Hemiclepsis marginata.* Brumpt has given no figures showing how this trypanosome develops into the pyriform parasites in the stomach of the leech; the description of these pear-shaped parasites in the leech clearly suggest a *Crithidia* similar to what we have seen in a species of *Glossiphonia.* Brumpt also states that the Herpetomonad forms of *T. granulosum* remain for months in the stomach of the leech; this fact again suggests a natural flagellate; we have found these flagellates remain for at least five months in the crop-diverticula of a leech. Brumpt states that the parasites in the leech eventually produce trypanosome-like forms which find their way to the foremost stomach coeca and proboscis sheath by the fifth day. With regard to this observation, Woodcock says, "these are the forms which are inoculated into the eel, becoming by simple elongation ordinary *T. granulosum* again." Anyone reading this statement might be led to believe that this has been proved by Brumpt, whereas this is by no means the case. Before it can be proved that the flagellates in the stomach of the leech were actually injected into the blood of the eel, Brumpt must demonstrate the change from one parasite to the other; it is also necessary to show that the leech has not got any unchanged *T. granulosum* in its proboscis sheath. Throughout these observations of Brumpt we can find no reference to the exclusion of a natural flagellate of *Hemiclepsis*; this is surely necessary.

Woodcock gives a detailed account of Miss Robertson's (1907) researches on a flagellate from the alimentary tract of *Pontobdella muricata*; with her, he regards it as representing the developmental forms of *T. raiae.* According to Woodcock, Brumpt has found that *T. raiae* does develop in *Pontobdella.* In an earlier paper we (1908) pointed out, after reading Miss Robertson's account of the life history of the flagellate of the leech, that the flagellate in question was most probably a true parasite of the leech. The structure of the adult flagellate and the method of development of its flagellum clearly suggest that this parasite is a true *Crithidia* —it is not at all unlike some of the tsetse fly crithidia—we have therefore named it *C. robertsoni.* All the evidence goes to show that this flagellate of *Pontobdella* is transmitted hereditarily. We can find nothing in Miss Robertson's or Brumpt's papers to show that it represents any stage in the evolution of *T. raiae.* Neither of these authors have attempted to exclude a natural flagellate of *Pontobdella,* and before this has been done

we do not see how it is possible to say *T. raiae* undergoes a true developmental cycle in *Pontobdella muricata*. Woodcock in criticising Miss Robertson's very cautious remarks regarding the process of conjugation of this flagellate boldly says, "it is more probable that conjugation itself takes place soon after the transfer of the parasites from one host to the other, *i.e.* after the arrival in the invertebrate; and that the ookinete form is the immediate result of the process." It is difficult to understand these wild speculations; such hypotheses, based as they are on erroneous conclusions, can hardly help forward this difficult subject. They are certainly not suggested by Léger's work on *T. barbatulae*, or Prowazek's (1905) work on *T. lewisi* in the louse.

Woodcock next shortly describes Keysselitz's (1906) work on the development of *Trypanoplasma borreli* in *Piscicola geometra*. According to that author, male and female gametes can readily be recognised in the blood of the carp, conjugation taking place in the leech and later giving rise to the three general types of Schaudinn. We (1909) have elsewhere shown that Keysselitz's developmental cycle cannot be accepted; he has not rigidly excluded a natural flagellate from the leech and his observations are therefore of little value. From his description we are unable to ascertain how *Trypanoplasma borreli* passes into the developmental forms in the leech. We therefore consider these parasites represent part of the life cycle of a *Crithidia* of the leech; the fact that Keysselitz found that leeches caught at large contained these flagellates assuredly suggests this. We would like to know whether any experiments were conducted to exclude a flagellate transmitted hereditarily.

In a recent paper Miss Robertson (1908) claims to have traced the development of a trypanosome of *Emyda vittata* in a leech. She states that the development is similar to what takes place on a slide (*sic*), and which is shortly as follows: "the trypanosome rolls itself up and the flagellum breaks free, but generally still remains attached at the kinetonucleus end. The flagellum is motile for a long time, but finally comes to rest, lying often in an untidy tangle round the creature. The trypanosome divides into two, the daughter individuals generally remaining more or less in contact; a further division into two occurs; the divisions in every case involve both the tropho- and the kineto-nucleus. The result of these divisions is a group of four often very irregular little creatures; they become pear-shaped and put out each a flagellum from their blunt ends and this gradually lengthens and becomes motile, but at first it is quite unable to move the body of this creature. The four little flagellates separate and move actively about." From this

very untidy description we gather that this trypanosome merely undergoes a process of multiplication exactly similar to that of *T. lewisi, T. rotatorium,* and others in cultures. We cannot regard this as a true developmental cycle and therefore the leech cannot be a true alternate host; no sexual cycle appears to be necessary. We note there is no mention of the possibility of the leech in question having a natural flagellate in its alimentary tract; perhaps Miss Robertson will show in her next paper that such a flagellate has been carefully excluded. If not we must doubt even this method of multiplication of *T. vittatae.*

All the above authors in their researches on the development of piscine and reptilian trypanosomes and trypanoplasmes in leeches have failed to exclude their natural flagellates; their descriptions also of the various life cycles are far from convincing. Some of the parasites appear to have a sexual cycle, while others have dispensed with the sexual process, and merely multiply asexually. Much of the work requires confirmation and reinvestigation. It is quite unjustifiable to draw any far-reaching conclusions from what we consider to be erroneous observations.

With regard to Prowazek's (1905) work we have recently criticised it and shown that there is no evidence to support the view that *T. lewisi* undergoes a developmental cycle in *Haematopinus spinulosus*; we consider Prowazek's developmental forms represent a part of the life cycle of a true *Crithidia* of the louse. Referring to the development of other mammalian trypanosomes in tsetse flies, Woodcock describes the work of Minchin, Gray and Tulloch. Minchin (1908) in a recent paper has drawn attention to the polymorphism exhibited by *T. gambiense* in the blood of a rat; two of the types he regards as sexual forms; there is however no proof that this view is correct. It has yet to be proved that these types represent male and female trypanosomes. We do not think there is the slightest evidence that these so-called male and female parasites conjugate soon after they pass into the stomach of an insect. Stuhlmann (1907), in his work on the development of *T. brucei* in *G. fusca,* has not described the male, female, and indifferent forms of the parasite of Nagana as depicted by Prowazek (1905); nor does he refer to the formation of ookinetes at the beginning of the infection of the fly. The first parasites he found in *G. fusca* were of the neutral type, which occurred in large numbers in the midgut of the fly. Woodcock thinks Stuhlmann probably missed some early essential phases of the development, for Léger found ookinetes of *T. barbatulae* in the leech eighteen hours after it had fed. According to Stuhlmann the indifferent para-

sites spread forward to the proventriculus where they became long forms, which Woodcock regards as corresponding with the slender forms of the flagellate of *Pontobdella muricata.*

In the proboscis of freshly caught tsetse flies Stuhlmann found little crithidial forms, which he regards as the stages of the parasite which are destined to pass back to the vertebrate. He however was unable to infect any vertebrate with *T. brucei* by injecting these parasites into them. Woodcock in trying to explain this discrepancy says, "There is some other, as yet unknown, factor or condition concerned in this perplexing question." We consider the perplexity arises from the fact that Stuhlmann failed to recognise the possibility, in fact certainty, of *G. fusca* being infected with a natural flagellate. Stuhlmann bred *G. fusca* from pupae and concluded the flies were free from infection, in other words, that hereditary transmission of the flagellates of tsetse flies does not take place. We can find nothing in Stuhlmann's paper proving this to be the case. Why did he not examine the alimentary tracts of the pupae for flagellates or their earlier stages? This should certainly have been done. Further, careful control experiments by feeding flies on clean animals should have been carried out and they should then have been examined for flagellates, but this appears to have been overlooked. All subsequent observers have accepted Stuhlmann's observations and have not attempted to exclude a natural flagellate of *G. fusca.* It is quite obvious Keysselitz and Mayer (1908) have fallen into this trap.

It will be remembered that Stuhlmann fed his raised *G. fusca* on animals infected with *T. brucei* and from two to four days later found that 80—90 % of the flies were swarming with flagellates. He then describes these flagellates as representing the cycle of development of *T. brucei.* A careful reference to his description and figures leaves us to imagine how *T. brucei* comes to develop into the flagellates of the fly. The masses of flagellates lining the intestinal epithelium clearly suggest a natural parasite, and to anyone who has studied a large number of these parasites this is at once evident. We therefore cannot accept this work as being accurate, and we regard his developmental forms as a true *Crithidia* of *G. fusca* which is transmitted hereditarily.

Minchin (1908), in his work on the development of *Crithidia grayi* in *G. palpalis,* describes three forms of this flagellate: (1) the ordinary type having a multiplicative function and giving rise to the swarms of parasites often found. It is usually of large size, and the blepharoplast shows great variability in its position; Minchin thinks this form most nearly

represents that in which *T. grayi* occurs in its vertebrate host (avian). (2) This type is constituted by slender, elongated parasites with well-developed undulating membrane and flagellum; it occurs chiefly in the hind gut of the fly. (3) Very narrow thin forms of a typical Herpetomonad structure with practically no undulating membrane. The parasites were found in the proctodaeum and produced the cysts of *T. grayi.*

In criticising the above work we would like to first point out that we do not agree with Minchin that this flagellate of *G. palpalis* has anything to do with a vertebrate trypanosome, but that it is a natural *Crithidia* of the fly. Minchin's hypothesis as to its origin is based solely on the habits of the tsetse fly; beyond certain observations on the feeding habits nothing is known of the behaviour of tsetse flies at their breeding grounds. In his recent paper Minchin (1908) dismisses Novy's original suggestion, that *T. grayi* is a fly parasite, by saying: "At the present time true trypanosomes are only known to occur in the blood of vertebrates, and in the stomachs of insects which suck the blood of vertebrates; hence, it is reasonable to assume that the insects in question obtain their trypanosomes from the vertebrates. When trypanosomes have been found in plant juices or stagnant waters it will be time enough to speculate on the possibility of blood-sucking insects obtaining them from such sources." Has Minchin studied this parasite, or any similar flagellate which encysts in the rectum of a blood-sucking insect, sufficiently to justify this statement? Did he, while in Uganda, endeavour to find the cysts of *T. grayi* in the faeces of *G. palpalis* in his fly cages, on twigs or even in plant juices or other places where the flies might deposit their excreta? We can find no reference to this in his paper. We would like to point out to him that we do not expect to find trypanosomes in plant juices or stagnant waters, but the *cysts* of a *Crithidia,* which is a very different thing. We have shown that these flagellate cysts are well adapted for an extracorporeal existence and that they can resist desiccation. We will here refer to one parasite which we have studied and which fully bears out our view. While making a study of *H. culicis* in *C. pipiens* and *C. fatigans* we found the encysted stages of the flagellate in the rectum of the mosquito as well as in its excreta. Exactly similar bodies were recovered from the alimentary tracts of the larvae of the insects. A study of the habits of the mosquito clearly explained the method of infection. The mosquitoes in one particular tank collected in large numbers in a dark corner just above the water were they subsequently laid their eggs. They were to

be found here in the day digesting the blood they had sucked the night before and undoubtedly passed their faeces into the water. Without actually finding the cysts of *H. culicis* in the water it was quite evident that the larvae ingested them accidentally while feeding on the surface of the water. We would draw Minchin's attention to these observations, for we believe, that if a careful study of the habits of the tsetse fly at its breeding grounds had been carried out, it would have been found that the flies accidentally ingest the cysts passed out in the faeces of other flies. A careful search should have been made in the alimentary tracts of a large number of specimens of *G. palpalis* for the cysts and the early stages of their development. Flies bred in captivity should have been placed in cages with wild flies to see if they became infected. One experiment carried out by Minchin suggests that this actually took place; he however interprets it in quite another way because the fly was unfortunately fed on a fowl. We are not even told whether this fowl had trypanosomes in its blood; this is a strange omission.

Before concluding that *T. grayi* undergoes the complicated cycle as suggested by Minchin, it would have been wiser to have first excluded a simpler and more natural life cycle such as we have suggested. Such complicated cycles are not common in nature. We believe that if *T. grayi* had been studied along the lines we have suggested the confusing number of forms described and figured by Minchin would have fallen into their natural places. We cannot agree with him in his description of the *Herpetomonad* forms. The flagellates he depicts as such are *certainly not* like a *Herpetomonas*; their anterior ends are drawn out along the flagella, clearly pointing to the presence of a narrow undulating membrane (see figures of *C. gerridis*). No *Herpetomonas* has this appearance. *T. grayi* we would then regard as a natural flagellate of *G. palpalis* belonging to the genus *Crithidia*; it is transmitted from one fly to the other by means of cysts which are accidentally ingested by other flies. The *Crithidia* of *G. fusca* on the other hand is transmitted hereditarily, and this accounts for the differences in the methods of development of the two parasites which have so perplexed Minchin, Stuhlmann and others.

Mode of Transmission of Trypanosomes by Tsetse Flies.

In an earlier part of this paper we pointed out that the study of the life cycles of trypanosomes was intimately connected with the elucidation of the methods by which they are transmitted in nature. In the course

of our criticism of the so-called developmental cycles of certain vertebrate trypanosomes in tsetse flies we have clearly shown that there is no evidence to support the views of those who maintain that these parasites of Africa undergo a true evolutionary cycle in the alimentary tracts of the flies. We firmly believe that Koch, Minchin, Stuhlmann, Keysselitz, Mayer and Roubaud have all confused these vertebrate trypanosomes of Africa with the natural *Crithidia* of the *Glossinae* and that therefore their conclusions are erroneous and misleading.

Owing to the great importance of knowing exactly how these pathogenic trypanosomes are transmitted to man and the lower animals, we now propose reviewing in detail the many observations that have been recorded. In order to make the subject quite complete it is necessary to begin with Bruce's (1896) experiments in Zululand. It will be remembered that Bruce on capturing some *Glossinae* (*G. morsitans* and probably *G. pallidipes*) kept them for a few days (exact time not being stated) and then fed them continuously on the same dog; in all, three such experiments were carried out. In one case five flies were fed on the same dog (1) every second day for nine weeks; in the second experiment, thirty flies were fed every day for ten days on the same dog (2) and in the third experiment fifty flies were fed daily for fourteen days on another dog. As a result of these three experiments Bruce found that none of the dogs became infected with *T. brucei*. It should be noted Bruce does not mention the length of time the flies were kept before they were fed on the dogs.

Bruce then carried out another series of experiments, this time feeding tsetse flies (presumably *G. morsitans*) on Zulu dogs infected with *T. brucei* and obtained the following results:

1. *Interrupted feeding.* In one case infection occurred after thirty-two feeds fifteen days after the first feed and in another case after twenty-four feeds with an incubation period of eight days.

2. *Twelve hours interval.* A dog became infected after nine flies had been fed 333 times.

3. *Twenty-four hours interval.* Twelve flies were fed on alternate days about 228 times and produced an infection in thirty-eight days.

4. *Forty-eight hours interval.* Twelve flies were fed on a dog for thirty-one days, seventy feeds were necessary before infection was produced.

5. Three days intervals gave negative results.

Bruce's experiments then clearly demonstrate that quite a small number of flies (*G. morsitans*) are able to infect clean animals immediately

after they had sucked the blood of an infected animal; and further that such infection can be produced up to an interval of forty-eight hours after sucking infected blood. Bruce also carried out one experiment (No. 225) in order to answer the question: "Is the tsetse fly capable of giving rise to the disease if taken out of the fly country into a healthy locality?" Bruce collected *Glossinae* in the low-lying fly country in the early morning, took them up to his camp on the top of Ubombo and immediately fed them on a healthy animal which subsequently became infected. It is presumed that these flies had already fed on an animal infected with *T. brucei* and that while they were feeding for the second time on a clean animal they were captured; yet when fed a third time on Bruce's clean animals on Ubombo they produced the infection. Remarking on these experiments Minchin (1908) says: "in our experiments on direct transmission, already recorded (*loc. cit.* p. 244), we found that if the fly, after feeding on an infected animal, were fed on two healthy animals in succession, only the first healthy animal became infected, not the second—that is to say, that by puncturing the skin of a healthy animal the proboscis is 'cleaned' for a second one. Hence, if the infection of *T. brucei* were only by the direct method, the flies caught off a healthy animal, in Bruce's experiment, should have been non-infective. The experiment seems to me, therefore, to indicate that in the case of *T. brucei* there is infection of a type other than the direct —that is to say, that cyclical infection occurs doubtless in addition to direct infection."

In a recent paper Kleine (1909) records some observations which seem to show that distant transmission, presumably after a developmental cycle, takes place in the case of *T. brucei* in *G. palpalis*. Kleine states, that as Nagana was not present in the Kirugo region, some sheep and a mule, which had been naturally infected by the bites of *G. morsitans*, were brought from a place seven days' march away; the infected animals were kept apart from the rest. Specimens of *G. palpalis* caught on the Mori river were fed for three days on a mule and two sheep infected with Nagana, and from the fourth day onwards they were fed daily on a fresh healthy animal.

From the 18th to the 24th day the flies fed on the same sheep (No. 30), and from the 25th to the 39th on the same ox (No. 2). The blood of the experimental animals was examined with negative results. On the 12th day after the flies were fed on ox No. 2 a preparation of its blood was examined and numerous trypanosomes were found; as a result of this discovery sheep No. 30 was examined and in its blood many parasites

were found. All the other animals remained healthy. Kleine remarks that from these experiments it is seen that flies, which for many days after the ingestion of *T. brucei* were not infective, afterwards became so, infecting first a sheep and then an ox. Presuming that Kleine's healthy animals were rigidly excluded from the bites of tsetse flies other than those he used, and that he was dealing with *T. brucei* throughout his experiments, it is clear that they are exactly similar to Bruce's experiment No. 225 (see Appendix for confirmation of Kleine's work by Bruce and his co-workers).

On the	4th	day	50	flies	fed	on sheep	No. 3.
„	5th	„	49	„	„	ox	No. 1.
„	6th	„	48	„	„	sheep	No. 4.
„	7th	„	48	„	„	„	No. 6.
„	8th	„	48	„	„	„	No. 26.
„	9th	„	46	„	„	„	No. 9.
„	10th	„	40	„	„	„	No. 10.
„	11th	„	40	„	„	„	No. 31.
„	12th	„	39	„	„	„	No. 12.
„	13th	„	39	„	„	„	No. 13.
„	14th	„	37	„	„	„	No. 14.
„	15th	„	37	„	„	„	No. 16.
„	16th	„	34	„	„	„	No. 17.
„	17th	„	34	„	„	„	No. 20.

G. palpalis is therefore able to transmit *T. brucei* twelve days after feeding on an infected animal. According to Minchin and Kleine this would show that during this interval *T. brucei* had undergone a developmental cycle in the alimentary tract of the fly and that the infection is of the cyclical type.

Kleine has however made no observations on such a developmental cycle in *G. palpalis*. It would be important in these experiments to ascertain whether there were any unchanged *T. brucei* in the alimentary tracts of the flies; Bruce has noted that *T. brucei* remains alive for five days in the alimentary tract of *G. morsitans* and its vitality appears to depend on the quantity of blood in the stomach of the fly. Kleine's flies were fed daily so that the parasites would here have had ample nourishment to keep them alive. This can however only be settled by further observations, but it must be remembered that *G. palpalis* itself is naturally infected with two *Crithidia—C. grayi* and *C. tullochi*. According to Stuhlmann and Minchin *T. brucei* undergoes a true develop-

mental cycle in *G. fusca*. Are we then to understand that this trypanosome undergoes its evolutionary cycle in both these flies? We think this is most improbable.

Cazalbou (1906) succeeded in infecting two dogs with *T. pecaudi*(?) by the bites of freshly caught *G. palpalis*; he also infected a cat with some of the same flies which had been starved three and a half days. He however does not record any observations to show what changes the parasites underwent in the flies during this period.

Bouet (1907) fed *G. palpalis* on dogs infected with *T. dimorphon*, and twenty-four hours later the fly was fed on a healthy puppy which became infected fifteen days later. Here again there is no evidence to show that *T. dimorphon* passed through a developmental cycle in the fly. Roubaud (1907) has also carried out some transmission experiments with wild *G. palpalis* and *T. dimorphon*. In one experiment he found that when the infected fly was merely allowed to bury its proboscis in the tissues of the uninfected animal and not to suck its blood no infection took place. When four *G. palpalis* were fed on a guinea-pig twenty-four hours after having sucked the blood of an infected white mouse, the guinea-pig became infected. In this experiment Roubaud believes the trypanosomes were regurgitated from the stomachs of the flies.

The discovery that certain tsetse flies are capable of transmitting the parasite of Nagana soon led to similar experiments on *T. gambiense* being carried out with these insects. Bruce, Nabarro and Greig (1903) have recorded a number of interesting observations in this connection. Tsetse flies (*G. palpalis*), which had first sucked the blood of a sleeping sickness patient, were fed eight hours later on a monkey; after 530 flies were fed during nine weeks the monkey became infected. A similar positive result was obtained with 509 flies. Nabarro and Greig (1905) have carried out similar experiments with *T. gambiense*; so also have Minchin, Gray and Tulloch (1906). Greig and Gray (1905) also tried to infect a monkey by using freshly caught tsetse flies; in one experiment 980 flies were fed for six and a half weeks before an infection was produced, while in another experiment 2299 flies were fed for eleven weeks on a monkey without infecting it. Dutton, Todd and Hannington (1907) also carried out a long series of feeding experiments with tsetse flies and *T. gambiense*; they however doubt whether their results are of much importance as their animals were only partially protected from the chance bites of insects.

It is quite evident from a perusal of all these records of transmission experiments that it is impossible to draw any exact conclusions, as they

have been carried out under such imperfect and varied conditions; for instance it is not certain in each case whether *T. gambiense* was the trypanosome under observation. Bagshawe (1909) has recently summarised the objections which apply to some or all of these experiments; we cannot do better than quote his remarks:

"(1) All were carried out with wild flies, and similar experiments at the same time and the same place showed that a certain number of such flies were infective when brought to the laboratory.

(2) The flies were in most cases fed on a healthy animal before being placed on the infected one with a view to their purging themselves of any trypanosomes they contained. But there is reason to suppose that such feeding might not have the effect desired; as Minchin points out, in Bruce's fresh fly experiments the flies were caught on healthy animals which it must be assumed they bit, and yet infection of the healthy experimental animal resulted.

(3) There is evidence that fresh flies retain the infection for more than a few hours. Cazalbou infected a cat with *T. dimorphon* after the flies had been starved for three and a half days.

(4) In some of the experiments attempts to transmit by fresh flies and by flies supposed to be infected in the laboratory were being carried on at one and the same time; this has been already pointed out."

There is however no doubt whatever that *G. palpalis* is capable of transmitting *T. gambiense* to man and it appears to be able to do so up to 22 days after it has sucked in the parasite. *T. brucei* also lives in the alimentary tract of *G. palpalis* and produces an infection after at least twelve days; in *G. morsitans* and *G. pallidipes* it can probably live longer than five days.

Schaudinn's work on the Haematozoa of the little owl.

We would hesitate to criticise Schaudinn's (1904) memorable work, but, owing to the fact that it is again inserted in an authoritative text-book, we feel bound to do so. In criticising it however we do not wish to throw any discredit on Schaudinn's work, we have the greatest admiration for it; it is not so much Schaudinn's mistakes we wish to draw attention to, but rather to emphasize the fact that *too* great weight has been attached to his conclusions. After all, Schaudinn's paper on the parasites of *Athene noctua* was only a preliminary report, and we feel sure that had he been spared he would have considerably modified

his views. We are told in a footnote by Woodcock (p. 239) that he has recently been able, after an arduous investigation on the Haematozoa of birds, to obtain the first definite evidence in support of Schaudinn's views. We shall criticise his recent findings later on.

It will be remembered that Schaudinn, after feeding *Culex pipiens* on the blood of the owl (*Athene noctua*) infected with *Halteridium danilewskyi*, claimed to have discovered the development of the intracellular parasites of the owl in the stomach of the mosquito. The *Halteridium* macrogamete, after being fertilised by the microgamete in the stomach of the mosquito, developed into an ookinete which became either a male, female or neutral trypanosome. These trypanosomes were then found in masses at times near the oesophageal end or at the junction of the ileum and colon. Schaudinn described complicated migrations of the parasites in the body of the mosquito, the parasites eventually developing into forms which were prepared to pass into the blood of an owl when this insect next fed.

Woodcock notes that the main criticism on Schaudinn's work is based on the probability of his dealing with at least four blood parasites: two free parasites, a trypanosome and a spirochaete (Töpfer) and two intracellular ones, *Halteridium* and *Leucocytozoon*. Woodcock however entirely omits from this list *Herpetomonas culicis*, *Crithidia fasciculata* and *Spirochaeta culicis*, merely mentioning them later on. We consider these are the most important parasites in connection with Schaudinn's work, and we believe that the other four blood parasites have played a small part in Schaudinn's complicated life cycles of *Halteridium* and *Leucocytozoon*. Schaudinn did not refer to his having excluded *Herpetomonas culicis*, *Crithidia fasciculata* or *Spirochaeta culicis* from his mosquitoes. We have shown that *Culicines* obtain the parasites *H. culicis* through the larva, so that in order to exclude this flagellate it is not only necessary to breed mosquitoes, but to be particularly careful in transferring the egg-rafts into water, where there is no chance of the larvae ingesting cysts passed out in the excreta of other mosquitoes. Schaudinn's mosquitoes were obtained at large at Rovigno, and we do not know whether they were caught as adults or hatched from larvae. It is not known at present how *Crithidia fasciculata* is transmitted from one insect to another; there is always the possibility that these particular flagellates may be transmitted hereditarily, and if this proves to be the case, the larvae would have to be bred from eggs laid by uninfected insects. *Spirochaeta culicis*, which we have recently found in the alimentary tract of adult *Culex pipiens* in

Cambridge, is probably also acquired by the larvae; in Madras we have seen these spirochaetes in great numbers in the alimentary tracts of the larvae of *Culicines.*

Bearing the above facts in mind, it is in the highest degree probable that Schaudinn's mosquitoes were infected with one or other of the flagellates we have referred to above, and, in addition, we can find nothing in his paper to show that he excluded *Spirochaeta culicis.*

Schaudinn found masses of flagellates rolled up in bundles and collected in various parts of the alimentary tract of *Culex pipiens.* Anyone who has studied these flagellates of mosquitoes knows that they are similarly found in bundles in different parts of the digestive tracts of the insects. Then again certain of Schaudinn's figures clearly depict the non-flagellate stage of *Herpetomonas,* and others, stages in the development of a *Crithidia.* Apart therefore from the possibility of his mosquitoes containing the trypanosome, spirochaete, halteridium and leucocytozoon of the owl, we believe the gravest source of error in Schaudinn's experiments lies in the fact that the insects may have also contained two flagellates and a spirochaete. Assuming then that his mosquitoes may have harboured no less than seven parasites, we are at a loss to understand how any investigator, even with Schaudinn's great technical skill and phenomenal powers of interpretation, could possibly follow any one of the parasites through a complicated developmental cycle; we know from experience that the resulting confusion would be exceedingly great.

It is generally stated (see Mesnil, *Bull. Inst. Past.* v. 3, 1905, pp. 363—367) that Schaudinn actually saw a *Halteridium* ookinete become a trypanosome presumably in the blood from the mosquito's stomach, and that the melanin granules helped to trace the stages in the evolution. With regard to this statement, we would like to point out that we have seen a leech *Crithidia* in its pre-flagellate stage containing melanin-like granules, and that if such a cell be studied, apart from its flagellate stage, it may quite well be mistaken for an intracellular pigment-containing parasite. It should therefore be borne in mind that these flagellates of invertebrates may contain pigment-like granules. Could it be possible Schaudinn was dealing with the pre-flagellate stage of a *Crithidia*?

We have noted above that in a footnote (p. 239) Woodcock states that he has at length obtained the first definite and unmistakable evidence in favour of one of Schaudinn's conclusions. As a result of his observations on a *Halteridium* of the chaffinch, he has no doubt that in

certain phases it becomes a little trypanosome, and in a recent paper he (1909) describes these observations. On examining the peripheral blood, heart blood, and smears of most of the organs of a chaffinch, between 1 and 3 a.m., they were found to be heavily infected with *Halteridium*, and less so with trypanosomes. Woodcock states that he was able to recognise three types of the *Halteridium*, male, female and indifferent; the latter being distinguished from the female type by its much lighter staining cytoplasm, and from the male type by a more compact and denser nucleus. He was not able to find any evidences of schizogony in the red corpuscles. Many of the *Halteridia* exhibited nuclear dimorphism; the nuclear elements separating into two constituents, a large body staining red with Romanowsky's stain, and a smaller structure, much denser, and staining darkly; the larger body Woodcock regards as the trophonucleus and the smaller as the kinetonucleus.

Actual passage from the halteridium stage to the trypanosome form could not be demonstrated with certainty, and Woodcock says, "They appear to be few and far between." He thinks this is due to the fact that there were very few trypanosomes in the blood of the chaffinch. In a smear of the heart blood he however found a body, which he thinks is an intermediate stage between the two parasites. This structure Woodcock considers is of the indifferent type, the two nuclei being in close contact. A delicate thread or line, which stained bright red, could be seen passing from a short transverse deep-staining band down part of the length of the body of the parasite, terminating in a definite granule. Woodcock thinks this thread represents the central spindle, described by Schaudinn, and which later becomes a flagellum. In a footnote Woodcock says he has found two or three of these cells. We must confess that the evidence brought forward by Woodcock in support of his view of this intermediate stage is exceedingly slender. It is not even clear that this parasite represents what was once a living halteridium, even in spite of the fact that the blood smear was made at once. We can find no proof whatever that this parasite is of the indifferent type, and that the chromatic mass represents the nucleus and blepharoplast in close contact. It is an unjustifiable assumption to say that the doubtful pink-staining strand represents a "central spindle." Surely Dr Woodcock cannot expect his readers to accept these statements without more rigid proofs[1].

In the bone marrow of a chaffinch he found small trypanosomes; he considers they have not arisen as the result of the division of larger

[1] In this connection see Mezincescu's (1909) recent work on Halteridium.

forms found in the peripheral blood, but have probably developed from halteridia. It is quite a well-known fact that small trypanosomes may be frequently found in the bone marrow of birds, and we think it most probable that these small forms may develop into the larger ones Woodcock figures. Woodcock believes that the great difference in the number of trypanosomes, as compared with the halteridia, is largely reduced when the sexual forms of the intracellular parasites are omitted. As a result of his observations, Woodcock concludes that "*Halteridium* is probably a stage in the life history of a trypanosome which has advanced a step further and become adapted also to the red blood corpuscles." It should be noted that Woodcock's results appear to be based on the study of stained films of the peripheral blood, heart blood and organs of a single chaffinch. We are not told whether the parasites were studied in the fresh condition, or whether more than one bird was examined. We are left in doubt also as to what the halteridium of the chaffinch might do in the stomach of its transmitting invertebrate; Woodcock's observations are based on such a small amount of material, that it is really difficult to ascertain what part of the life cycle of the parasite he was dealing with.

As far as we are aware, no other observers have noted this so-called nuclear dimorphism in halteridia in the blood of birds. We have examined many specimens of the male and female elements of the halteridium of *Athene brama* from the blood of the owl, but we must admit we have never seen a true blepharoplast in any of them. It is by no means proved that the structure Woodcock describes as a kinetonucleus is a true blepharoplast; as far as we can gather, this view is chiefly based on its staining reactions.

We are unable to accept Woodcock's explanation of the difficulty in finding so few examples of the intermediate stages, between the halteridium and the small trypanosomes. Surely, if this parasite is to become a trypanosome, and provided a sufficient number of birds are examined, there ought to be no difficulty in finding numerous examples of all the stages in this very important change. The single parasite, which Woodcock depicts as illustrating one of the changes towards the formation of a trypanosome, may represent anything, as it is lying free in the plasma. We can find no proof that it is destined to become a trypanosome. We therefore think it is extremely doubtful whether the trypanosome of the chaffinch has any connection with its halteridium; Woodcock's observations certainly do not support any such conclusion. We would like to see a more exhaustive study of fresh preparations of a large number of

birds, as well as the development in an invertebrate. Woodcock does not appear to have touched on this side of the question; after all, this is where Schaudinn studied halteridium. If the difficulties in this country are too great, would it not have been better to have investigated this problem at Rovigno?

We do not wish to discourage Woodcock in his endeavours to confirm Schaudinn's conclusions, but we must say this last piece of work is most unconvincing; it is to say the least of it premature. Schaudinn's work on *Halteridium* therefore, in our opinion, remains as yet unconfirmed. It has certainly had a far-reaching effect, and we would now like to see the problem investigated by an international commission, carrying on its work at Rovigno.

In the seventh section Woodcock deals shortly with insect flagellates and their bearing on the evolution and phylogeny of trypanosomes. Léger's (1902) definition of the genus *Crithidia* is again introduced, as well as his work on certain *Herpetomonads*. We have shown that this definition of the genus *Crithidia* is inaccurate, as it is based on the structure of the immature stages of *Crithidia fasciculata*; many Herpetomonads have a very similar appearance. Woodcock's description of these important flagellates is extremely inadequate and out of date; the two genera are throughout confused, as they are in Léger's original papers. Such a statement, as the following, will show that Woodcock's knowledge of these parasites is mainly second hand: "These forms (*Herpetomonas*) are mostly parasitic in invertebrates which do not suck blood." We know of at least two which are parasitic in blood-sucking insects, Herpetomonads of *Culicines* and *Stomoxys*.

Woodcock next goes on to discuss the relation between these flagellates and the trypanosomes of vertebrates. It will be remembered that Léger (1902) expressed the opinion that the *Crithidia* of mosquitoes was most probably but a stage in the development of a Haemoflagellate, and that many more of these flagellates may eventually prove to be connected with blood parasites. Novy has however expressed the opinion that these insect flagellates are distinct from the blood trypanosomes. Ross (1906) has pointed out that he found the *Herpetomonas* of *Culicines* in the insects before they had fed on blood. We (1907) have recently been able to confirm these old observations of Ross, and have further pointed out that the larvae acquire the infection by ingesting cysts of the parasites passed out in the excreta of the adult insects.

In summing up these opposing views, Woodcock attaches the utmost weight to the flagellates of the tsetse flies, which Minchin and others

regard as stages in the development of vertebrate trypanosomes. We have shown above that the proof of this is wanting, and that there is also no proof to support the view that certain piscine trypanosomes undergo their development in the alimentary tracts of leeches. In all the instances we have quoted above, natural flagellates of the various sanguivora have not been excluded. We have no hesitation in saying that *Herpetomonas culicis* and *Crithidia fasciculata*, studied by Novy and his collaborators (1907), are in no way connected with an avian trypanosome. The American observers only studied the flagellate stages of the parasites in the mosquitoes and in test tubes. Had they however investigated their life cycles, as we have, we have no doubt that they would have found conclusive evidence to show that these flagellates are entirely limited to their insect hosts. We therefore cannot agree with Woodcock that any of these insect flagellates are connected with avian trypanosomes. It is quite premature to say that some of them may have developed a trypanosomal condition as an adaptation to the food of these sanguivorous insects, without being able to live in the blood of the vertebrate. The study of these flagellates in test tubes, and more especially partial descriptions of their life cycles, have largely contributed to the many erroneous views regarding their structure and relations.

In his first article (1906) on the Haemoflagellates Woodcock expressed the opinion that trypanosomes had originated from an ancestor parasitic in invertebrates. He however now believes that the trypanosomes which have the vertebrate for their primary host are Heteromastigine forms, *i.e.* are derived from a bi-flagellate Bodo-like type; those parasitic in invertebrates he believes have originated from a Monadine ancestor. Woodcock states that in bringing forward this view he has been mainly influenced by the intestinal *Trypanoplasmata*, and by Minchin's observations on *Crithidia grayi*. As so little is known of the intestinal trypanoplasmata, we think it is dangerous to draw important conclusions from our present knowledge of these forms. Are they true trypanoplasmata? We do not know this for certain. We have pointed out above that Minchin's hypothesis, regarding the life cycle of the flagellate of *G. palpalis*, is based on an incomplete study of its life history, as well as that of the tsetse fly, and that it is most improbable; in our opinion *C. grayi* is a true parasite of *G. palpalis*. Woodcock further thinks sufficient weight has not been attached to the fact that the majority of invertebrates, which harbour trypanosomes (according to our view crithidia), are blood-sucking insects. A large number of *Rhynchota* and flies which are *not* blood-suckers harbour typical *Crithidia*.

How then can these invertebrates be second or intermediate hosts of various vertebrate flagellates? As far as we know there is no proof to show that the common house fly has descended from a blood-sucking ancestor, and that *H. muscae domesticae* and the new flagellate we have found in its malpighian tubes have been derived from haemal forms. The same may be said of *Sarcophaga* and its flagellates, *Lucilia* and a host of others.

Woodcock in his remarks on the diphyletic origin of the trypanosomes refers the reader to his original article (1906, pp. 270—278), where he discusses the whole question at length; we shall now examine his arguments in detail. Before however doing so, it is necessary to understand our position with regard to this difficult subject.

We consider Woodcock's *Trypanomorpha noctuae* (Schaudinn's *T. noctuae*) is nothing more than *Crithidia fasciculata* and possibly *H. culicis*, and that the *Crithidia* and *Herpetomonas* of blood-sucking arthropods and leeches are true parasites of these invertebrates, and therefore do not represent part of the life cycles of vertebrate trypanosomes. In support of his views Woodcock lays particular stress on certain biological and morphological conditions. Under the first heading he says that the sensory extremity of a Haemoflagellate is its anterior end, and that *T. ziemanni*, in its resting stage, attaches itself and penetrates a leucocyte by its non-flagellate extremity. The parasite referred to here is *Haemamoeba ziemanni*, Laveran, which Schaudinn regards as being but the stage of a *Spirochaeta*. We believe that Schaudinn confused this intracellular parasite of the owl with the flagellates of *Culex pipiens*, and more particularly with *Spirochaeta culicis*. In any case we do not understand what connection this *Haemamoeba* has with a Haemoflagellate, and it is by no means proved that it is a spirochaete. *Herpetomonas* and *Crithidia* we know always attach themselves to the intestinal epithelium of their hosts by their flagella; on the contrary it is not known by what means the trypanosomes and trypanoplasmes attach themselves. It would be important to know how *Crithidia* penetrate the eggs of their hosts and how *Endotrypanum schaudinni* penetrates the red corpuscles of *Cholaepus didactylus*. Granting any of the points on the methods of attachment brought forward by Woodcock, it is however hardly justifiable to draw any far-reaching conclusions from a few doubtful observations.

With regard to the process known as agglomeration, we have pointed out above (p. 97) that a clear distinction should be drawn between the true rosette and a mere agglomeration of adult flagellates; the former is

a developmental process, while the latter is brought about by certain unknown changes in the medium in which the parasites live. *Herpetomonas* and *Crithidia*, as far as we have studied them in the intestinal juices of insects, agglomerate in their adult stages by their flagellar ends; and it is a physiological process. Adult *Trypanosomes* however collect together by their aflagellar ends and this change takes place under unfavourable conditions; it is doubtful whether it naturally occurs in the blood of vertebrates. It is therefore most probably a pathological process. In the case of *T. lewisi* it is well seen when the serum of a rat, inoculated two or three times with this trypanosome, is added to blood rich in parasites. Laveran and Mesnil consider this process is brought about by a specific agglomerin. The agglomeration of *Herpetomonas* and *Crithidia* in the intestinal fluids of insects is therefore by no means comparable with the agglomeration of vertebrate trypanosomes under unfavourable conditions. Can we then attach any great importance as Woodcock does to the different methods of agglomeration?

The process of conjugation is hardly worth mentioning as there is not a single instance in which it has been described from Haemoflagellates; the so-called examples of conjugation, *T. lewisi* and *T. brucei* (Prowazek), represent something quite distinct, for example in the case of *T. lewisi* from the louse they are undoubtedly *Crithidia* dividing unequally.

The majority of trypanosomes we have studied travel forwards by their flagellar ends, except perhaps the very stout forms of *T. rotatorium*. It should however be remembered that we are only able to observe the movements of these flagellates under artificial conditions; the very best instruments we can employ can hardly replace the conditions in the blood of the host. Are we to understand then that the slow crawling movements of certain trypanosomes on slides, when they move by their aflagellar ends, are their normal methods of locomotion? We are not sure of this and we would hesitate to attach any great importance to it.

Considering then more closely all the biological data brought forward by Woodcock to support the view, that the non-flagellate end of a trypanosome is the sensitive end, we find that the majority of his arguments are open to grave criticism, and the only point we might concede him is the possible progression of some trypanosomes by their aflagellar ends under unfavourable conditions.

Under the heading "morphology" Woodcock lays stress on the cell-vacuole situate in the aflagellar end of certain trypanosomes, *T. lewisi* and *T. gambiense*. Are we to consider this a normal cell constituent, and does it represent the (original) contractile vacuole of an ancestral

Haemoflagellate? Is it homologous with a similar structure in *Trypanoplasma* and *Herpetomonas*? We would like to ask Woodcock whether he has any definite evidence to support his contentions? Surely it is gratuitous assumption to say what he does about it, and to attach such importance to it. We would prefer to leave it alone at present, till we know something more about it. The same may be said regarding the chromidia of trypanosomes and allied flagellates. We do not know for certain that their position in a parasite is of much importance.

All the data, both biological and morphological, brought forward by Woodcock, appear to us, after detailed examination, to be of very little importance, and some of his assertions are certainly incorrect; we therefore consider that they do not add any weighty support to his view on the diphyletic origin of the trypanosomes.

Brumpt and Léger's hypotheses.

It will be convenient here to consider some of the other hypotheses on the origin of the Haemoflagellates of vertebrates. Brumpt (1908) in a recent paper has summarised his views on the origin of these parasites. He first refers to Chatton and Alilaire's (1908) recent discovery of a trypanosome from the malpighian tubes of *Drosophila confusa*. We are not sure that this flagellate is a true trypanosome, for the reason that both *Herpetomonas* and *Crithidia* may exhibit an appearance very like this parasite (see Donovan, 1909). We (1909) have recently figured this stage in a *Crithidia*, *C. ctenophthalmi*, from the alimentary tract of *Ctenophthalmus agyrtes*; in this particular stage (*post-flagellate*) this flagellate is not at all unlike some trypanosomes. We have seen Herpetomonads exhibit a somewhat similar appearance, and Roubaud (1908) has figured this stage in two *typical* Herpetomonads, *H. mirabilis* and *H. mesnili*. We would like to point out here that this trypanosome-like phase is seen in the *post-flagellate* stages of these flagellates, and we believe the migration of the blepharoplast to the posterior end of the cell is due to the degeneration of the root of the flagellum and the subsequent liberation of the blepharoplast. We note Roubaud (1908), found these stages of *H. mesnili* in the posterior intestine of a species of *Lucilia*; he has however made no attempt to trace out the life cycle of the parasite. At present therefore we cannot accept the statement made by Brumpt that Chatton and Alilaire's recent discovery confirms his hypothesis on the origin of the Haemoflagellates. Brumpt says, "*Ces Flagellés seraient des vulgaires parasites intestinaux d'invertebrés*

inoculés fortuitement par ces derniers aux vertébrés, au milieu sanguin desquels ils ont pu s'adapter." Léger (1902) has also advanced a similar hypothesis, that the *Crithidia* and *Herpetomonas* of biting insects are perhaps but stages in the evolutionary cycle of vertebrate trypanosomes.

Brumpt bases his hypothesis on what he considers to be the development of certain fish trypanosomes in leeches; we have fully criticised his researches on the evolution of these trypanosomes in leeches, and have clearly shown, that as he has not rigidly excluded a natural *Crithidia* of the leeches, his work on the fish trypanosomes cannot be accepted as being accurate.

Brumpt goes on to say "*A un point de vue général, mes études sur l'évolution des Flagellés des Poissons montrent que les cultures artificielles des Trypanosomes sur gélose au sang représentent certainement le cycle évolutif dans l'hôte intermédiaire à sang froid* (*Glossines, Puces, Taons, etc.*); *elles expliquent également pourquoi ces cultures réussissent mieux à froid qu'à chaud.*"

This statement is wholly inaccurate, as there is no evidence to show that the artificial multiplication of trypanosomes on blood agar "*certainly represents*" the evolutionary cycle in a cold-blooded intermediate host. It has not yet been proved that certain vertebrate trypanosomes of Africa, *T. brucei* and *T. gambiense*, undergo any evolutionary changes in tsetse flies (see p. 103 and Appendix); *T. lewisi* certainly does not undergo the same multiplicative changes in fleas as it does in blood agar; we have ourselves investigated this question. There is also no evidence to show that a vertebrate trypanosome undergoes a developmental cycle in horse flies; we have recently made a complete study of a *Crithidia* in a Tabanid, and we know it has no connection with any trypanosome, but is a true parasite of the fly. Brumpt further states, "*Actuellement, je suis bien convaincu qu'elles* (*la mouche tsétsé*) *sont des hôtes intermédiaires au même titre que les Hirudinées.*" Here again we have shown that the tsetse flies cannot be considered as true intermediate hosts of pathogenic trypanosomes, for the simple reason that there is no evidence to show that these trypanosomes undergo any development in the flies; the so-called developmental forms are certainly natural *Crithidia* of the flies. Again there is no proof that the trypanosomes after undergoing a developmental cycle in the flies pass to the proboscis and are then inoculated into a vertebrate, when the fly sucks its blood; the same may be said for the flagellates of leeches. All the evidence, at present to hand, appears clearly to show that the pathogenic trypanosomes of

Africa are transmitted mechanically by the tsetse flies, and this probably also holds good for leeches and the trypanosomes they transmit.

We have fully criticised Brumpt's work on the development of *T. granulosum* in *Hemiclepsis marginata* and pointed out that, as he failed to exclude a natural flagellate of the leech, his work cannot be accepted as being altogether correct. From our present knowledge we are justified in saying, that the trypanosomes of vertebrates do not undergo any developmental (*sexual*) cycle in invertebrates; how then can they be considered as representing primitive intestinal or entero-coelomic parasites of invertebrates which have become partially or secondarily adapted to the blood of a vertebrate?

Brumpt concludes his paper with the following remarkable statement: "*Enfin, en juillet* 1907, *je démontre la transmission héréditaire normale de Trypanosomes et de Trypanoplasmes chez les Sangsues, tout à fait en dehors du parasitisme d'un hôte vertébré. Ce qui démontre d'une façon définitive que la maladie sanguine du vertébré n'est qu'un accident, que l'adaptation d'un parasite intestinal banal d'invertébré.*" In our opinion Brumpt is here obviously confusing the natural *Crithidia* of leeches, which are transmitted hereditarily, with the trypanosomes they may ingest. We know of no definite experiments showing that trypanosomes of vertebrates may be transmitted through a sanguivorous invertebrate to its progeny. Brumpt (1907) has recorded certain observations on the hereditary transmission of what he considers to be trypanosomes and trypanoplasmes of vertebrates in leeches. The first experiments described are those with *Helobdella algira* which transmits *T. inopinatum*. One leech infected with *T. inopinatum* gave birth to a number of embryos, two out of every ten of which were infected. Four of these embryos were allowed to arrive at maturity, and two of them laid eggs, which were not infected, while the remaining two gave birth to infected embryos, one showing five out of ten and the other nine out of ten with parasites. The trypanosomes were generally found in the sheath of the proboscis, also in the caeca, but never in the intestine. The parent leech referred to above was presumably infected with flagellates, but we would like to know on what grounds Brumpt regards these as being developmental forms of *T. inopinatum*? No figures of these parasites are published, and no mention is made of the leech being infected or not with a *Crithidia*; Brumpt has apparently overlooked such a parasite. Further, no experiments are recorded to demonstrate whether or not the infected embryos of the first generation are capable of transmitting *T. inopinatum* to healthy frogs; such experiments are

necessary. A certain percentage of the second generation of leeches were also infected with flagellates, and these again were regarded as stages of *T. inopinatum*, yet we can find no proof that this is actually the case. In a species of *Glossiphonia* we found that about 80—90 % of the embryos from an infected parent had *Crithidia*; these flagellates were in no way connected with a frog trypanosome, nor was it possible to transmit them to healthy frogs. We therefore believe that Brumpt has fallen into the error of describing a natural *Crithidia* of *H. algira* which is transmitted hereditarily. We can well understand, that as a certain percentage of embryos of this leech are not infected, all their progeny would remain clean, and that although they may be fed ever so many times on infected frogs, they will themselves never become infected; in our opinion this does not indicate that the leech is refractory, but that *T. inopinatum* does not undergo any developmental changes in the leech. In one experiment forty uninfected leeches were fed on infected frogs for ten days, and then isolated for seven days, after which they were placed on a clean frog which later became infected. This experiment in our opinion only shows that *T. inopinatum* may be transmitted after at least seven days[1]; we would like to know if there were any unchanged trypanosomes (*T. inopinatum*) in the sheath of the proboscis of the leeches.

In the case of *Helobdella stagnalis* Brumpt found trypanoplasmata abundant in the proboscis of specimens of this leech; a large percentage of the embryos of an infected parent were themselves infected. We are left in doubt as to the origin of this trypanoplasm, and would like to know whether it is a true *Trypanoplasma* or a *Crithidia* of the leech transmitted hereditarily. Brumpt has not published any figures of the flagellate.

Brumpt has examined some hundreds of embryos of *Hemiclepsis marginata* descended from adults infected with various species of trypanosomes and trypanoplasmes of fresh water fish, but he was never able to demonstrate hereditary transmission. Similar results were obtained with *Piscicola geometra.* Is it possible that the flagellates of these two leeches are transmitted in some other way? In every instance Brumpt has failed to exclude a natural flagellate of the leeches he was dealing with; surely this is a grave source of error. Now that it is known that these flagellates commonly occur in leeches, it is first necessary to exclude them before it is possible to trace the development of any other parasite which may be ingested by these invertebrates.

[1] This experiment at once recalls Kleine (1909) and Bruce's recent work.

We cannot then agree with Brumpt that the haemoflagellates of vertebrates are common intestinal parasites of invertebrates.

We have noted above that Léger has advanced much the same idea, and he believes that the flagellates of vertebrates were once true insect forms which have gradually become adapted to the blood ingested by invertebrates, and have in process of time become transferred to vertebrates. This is a very simple and convenient hypothesis, but unfortunately there is at present no proof in support of it; we do not know of a single vertebrate trypanosome which passes its sexual cycle in an invertebrate, that is, its life cycle does not consist of an alternating blood and gut parasitism.

Where then have the trypanosomes come from? In studying the methods of development of *T. lewisi* and *T. rotatorium* in their *vertebrate* hosts, it is clear that they exhibit changes very similar to *Herpetomonas* and *Crithidia*; according to Salvin-Moore and Breinl *T. gambiense* and *T. equiperdum* also exhibit such stages. These facts suggest that the ancestor of the present true trypanosome of the blood of vertebrates was a crithidia-like flagellate. The interesting question is, was this crithidia-like ancestor a blood parasite or an intestinal form? At present it is impossible to answer this question, as no true trypanosome has been found in the intestinal tract of a vertebrate or invertebrate. The only instance of what may be a trypanosome has been recently described by Chatton and Alilaire (1908) from the malpighian tubes of a non-biting fly *Drosophila confusa*. We do not consider this parasite is a true trypanosome, because the authors have not worked out its complete life cycle, and may quite well have mistaken a particular stage in its life history for a trypanosome (see p. 119). Even admitting that this parasite is a true trypanosome, its occurrence in the intestinal appendages of a non-biting fly is exceedingly difficult to explain.

The greatest obstacle in the way of understanding this group of blood parasites is the fact that, although they have a common morphological structure, they exhibit great variation in their length, breadth and other minor details, and most important of all we know practically nothing regarding their life cycles. An important advance however in the study of these parasites has recently been made by Salvin-Moore and Breinl; these authors have brought forward observations on certain trypanosomes, which suggest that they pass the most important part of their life histories in the blood and organs of their vertebrate host. Let us take *T. lewisi* as an example, this flagellate (see Nuttall, 1909) can be readily transmitted to white rats by means

of fleas (*C. fasciatus* and *C. agyrtes*) by placing on them a number of these insects, which have recently been removed from an infected wild rat. Now we know this trypanosome does not undergo any developmental changes in the intestinal tracts of the two fleas, and that the parasites are most probably transmitted mechanically after finding their way to the proboscides of the insects.

In from six to seven days after the fleas have been placed on a rat, if the experiment is going to prove successful, the blood of the rat will be found to contain large numbers of young flagellates. Some of these are quite round and have short or long flagella, and are exactly like certain *Crithidia*, especially those undergoing division (see Laveran and Mesnil's (1904) excellent figures illustrating these changes in *T. lewisi*). The multiplication rosette of *T. lewisi* is also very suggestive of a *Crithidia* (see figures 6, 7 and 8 Laveran and Mesnil (1904)); the flagella of the developing parasites are directed externally and each flagellum at an early stage is attached to the body of the parasite (compare the above figures with those of *C. gerridis*). *T. rotatorium* also exhibits similar appearances in its developmental stages in the blood of frogs. We do not wish here to include the cultural forms of a number of trypanosomes, as we do not consider they represent the true developmental cycles of the parasites, but are rather pseudo-developmental forms; certain trypanosomes readily exhibit these changes, when they are cultivated on artificial media, such as blood agar; why they do so we cannot explain. We think then these facts suggest that the two trypanosomes (*T. lewisi* and *T. rotatorium*) have descended from crithidia-like ancestors. So far the majority of *Crithidia* have only been found in the intestinal tracts and appendages of blood-sucking and non-blood-sucking arthropods and leeches, but quite recently Wenyon (1909) has recorded a flagellate from the blood of a snake (*Erythrolamprus aesculapii*), which is very like some *Crithidia*; and again the flagellate which Brimont has recently found in the red blood corpuscles of *Cholaepus didactylus* may quite well prove to be but a stage of a *Crithidia*. We may then, in the case of these parasites, be dealing with two haemal forms, which perhaps exhibit the peculiarities of ancestral haemoflagellates. The fact that one of these parasites penetrates the red blood corpuscles of a vertebrate, suggests that it may probably be the more primitive of the two. We would then suggest that the ancestors of the present trypanosomes may probably have been intraglobular crithidia-like forms. We will leave this question at this point, as it is unwise with our present limited

knowledge to speculate further. When we know more of the life histories of the trypanosomes and especially of such forms as have recently been described from snakes and from the two-toed sloth, we may be in a position to formulate a more definite hypothesis regarding the phylogeny of the Haemoflagellates. Before leaving this subject however we wish to emphasize the fact that the present *Herpetomonas*, *Crithidia* and *Trypanosoma* are *distinct* forms, and that as far as we know one *does not* become converted into the other. Because a trypanosome may exhibit certain stages in its development like a *Crithidia*, we are not justified in calling it a true *Crithidia* and *vice versa*; exactly the same may be said regarding *Herpetomonas* and *Trypanosoma*.

The eighth section of Woodcock's article is devoted to the systematic consideration of the Haemoflagellates; here we find that he divides the sub-order *Monodina* into the single family *Trypanomorphidae*. We have shown above that the genus *Trypanomorpha* is almost certainly *Crithidia fasciculata* of Léger, so that this new genus can hardly be retained. Woodcock divides the sub-order *Heteromastigina* into one family in which he places the genera *Trypanoplasma*, *Trypanophis* and *Trypanosoma*; we are left in doubt as to where the important genera *Herpetomonas* and *Crithidia* come into this classification as no mention is made of them here.

We have pointed out above that the orientation of the body, and the biological features associated therewith, which Woodcock has used as important diagnostic characters in support of his classification, are very faulty, and that therefore this grouping of these flagellates is open to criticism. In the present state of our knowledge, it would have been wiser to have refrained from making new families, and genera, and to have been contented with a much simpler and more natural classification. We would prefer to group the Haemoflagellates and allied forms as follows:

Class	**MASTIGOPHORA.**
Sub-class	LISSOFLAGELLATA.
Order	MONODIDEA.
Sub-order	Monodina.
Family	*Trypanosomatidae.*
Genus	*Trypanosoma.*
„	*Trypanoplasma.*
„	*Crithidia.*
„	*Endotrypanum.*
„	*Herpetomonas.*

Woodcock gives a short account of some of the more important trypanosomes, grouping them together under the heading of their various hosts. We note that such important parasites as *T. evansi*, *T. equiperdum* and *T. theileri* are only just mentioned; we would like to have seen these and other lethal forms described fully.

Woodcock's appendix (A) is devoted to a consideration of the Leishman-Donovan-Wright-bodies, but it does not contain anything new regarding the parasite of Kala-Azar. We fail to understand what connection Wright has with this parasite, unless it is that Woodcock regards it as identical with the parasite of Oriental Sore. We note that Woodcock considers these two parasites are closely related on the one hand with *Herpetomonas*, and on the other with the *Piroplasmata*. We need hardly say that we do not consider there is any evidence to support the view, that the parasite of Kala-Azar is in any way connected with a *Piroplasma*. It has yet to be proved that the *Piroplasmata* have a true blepharoplast analogous to that seen in *Herpetomonas*, *Crithidia* and *Trypanosoma*.

Through the kindness of Professor Nuttall we have had the opportunity of examining Breinl's (1908) recently described flagellate stages of *P. canis*, and we have no hesitation in saying, that they are quite unlike the characteristic flagellates of *Herpetomonas*; indeed we are doubtful whether they represent true flagellates. With regard to Kossel and Weber's observations (see Schaudinn, 1904) on the flagellates found in smears of the intestinal contents of ticks, fed on cattle infected with *Piroplasma*, we would suggest that they were probably only flagellates of the tick, such as are known to occur in *Rhipicephalus sanguineus* and *Haemaphysalis flava*.

Professor Nuttall, who is at present investigating *Theileria* (*Piroplasma*) *parva*, has kindly permitted us to state, that cultural experiments with this parasite, up to 149 hours, have so far yielded negative results. We (1907) have elsewhere suggested that Miyajima's (1907) observations on cultures of *T. parva* of Japanese cattle are probably erroneous, in spite of the fact that he carefully guarded against the possibility of his cultures being infected with trypanosomes of the cattle. Miyajima's figures of the seventy-two hour cultures show typical trypanosomes. How the diminutive *T. parva* comes to develop into these huge parasites is not explained; we would like to see this work confirmed by other observers.

We (1908) have recently described a *Herpetomonas* which is identical in appearance with the parasite of Kala-Azar, and further we have pointed out that these herpetomonads exhibit the same stages in their

life histories as the human parasite does in man, and in the bed bug *Cimex rotundatus*. We therefore see no reason why this flagellate should be placed in a distinct genus (see also remarks in recent paper by Patton, 1909).

Woodcock makes no reference to Nicolle's (1908—1909) recent work on *Herpetomonas infantum*, the causal agent of Algerian herpetomoniasis. Nicolle has studied ten cases of this disease in Tunis, the characteristic symptoms of which are, irregular fever, anaemia, general emaciation, hypertrophy of the spleen and oedema. All the cases occurred in young children from several months to five years old; Indian herpetomoniasis on the other hand is common both in children and adults of all ages. Nicolle has only been able to find the parasite once in the peripheral blood of a patient; this contrasts markedly with Donovan's observations in Madras, where Kala-Azar is diagnosed solely by examinations of the peripheral blood. Nicolle (1908—1909) has succeeded in reproducing the Algerian disease in dogs and monkeys, by inoculating them with infected tissue; he has also cultivated the parasite on blood agar, not only from the spleens of diseased children, but also from naturally infected dogs. He however failed to reproduce the disease in dogs and monkeys by inoculating them with pure cultures, and concluded that the cultural forms had probably lost their pathogenicity. Novy (1909) has however recently succeeded in infecting a dog with *H. infantum* by inoculating it with 270 tubes of blood agar cultures in 15 inoculations extended over a period of 161 days. We would like here to draw attention to a point which we think both Nicolle and Novy have overlooked. It is well known that in artificial cultures of these human parasites many of them never develop into flagellates, but some remain for many days, even months, unchanged; we have ourselves seen this in cultures of the parasite of Kala-Azar. Now Nicolle and Novy's observations show that a very large number of *cultural forms* (flagellates) have to be inoculated before the disease can be reproduced in dogs, whereas a *small quantity* of blood containing the *undeveloped parasites* readily produces it. Could it not then be that in Novy's cultures there were some *unchanged* (our pre-flagellate stage) parasites, and that by inoculating a sufficient number of these he was able to reproduce the disease? Novy however does not state whether his cultures contained unchanged forms in addition to the flagellates; we would draw his attention to this point. The reason why we suggest this, is because we have demonstrated beyond any doubt that all Herpetomonads, in order to pass from one host to another, must return to their non-flagellate (post-flagellate) stages. In the case of

those species entirely limited to insects, this stage takes place by the parasites encysting, either in the rectums or ovaries of their hosts. We believe therefore that these human parasites, in order to be reintroduced into man, must be inoculated in their non-flagellate stages, and it is most probable that Novy was dealing with some of these unchanged forms in his cultures. This clearly explains the reason why it is necessary to inoculate such large numbers of *flagellates* in order to produce the disease, while a very small quantity of splenic blood containing the pre-flagellate stages of *H. infantum* readily produces the disease in dogs and monkeys.

As a result of his observations on the canine form of the disease, Nicolle claims that this animal is probably the source of the parasites which are inoculated into children through the intermediary of some blood-sucking insect. It is however by no means proved that Nicolle is dealing with a single species, the canine parasite may possibly be quite distinct from the infantile form; this can only be settled by finding out the invertebrate hosts of the parasites. With the infection in an animal like the dog, there ought to be no difficulty in carrying out feeding experiments with likely blood-sucking insects, in order to find out in which particular one the flagellate stage occurs. Similar experiments should be carried out with the human disease, and then once having found out the right insect, transmission experiments could be conducted on dogs and monkeys. So far as we are aware such experiments, as we have mentioned, have not yet been carried out.

Recently we (1908) inoculated dogs with the fresh virus of Indian herpetomoniasis with negative results, and we pointed out that so far *H. donovani* has not been found in Indian dogs; these observations confirm our view that this parasite is distinct from the Algerian form. We have shown that many herpetomonads are identical in their non-flagellate stages, and that if this stage alone is studied it is impossible to distinguish between distinct species; it is only by studying the complete life cycles of these parasites that this can be accomplished.

The parasite of Oriental Sore in its non-flagellate stage is also identical with the similar stages of the above parasites. Nicolle (1908) has succeeded in cultivating the Algerian form of Oriental Sore on blood agar and shows that it does not differ to any great extent in its *cultural* forms from those of *H. infantum.*

Row (1909) has recently cultivated the parasite of Delhi boil, and finds that though its method of development is very similar to that of *H. donovani,* it differs in some structural details. We have not seen

Row's figures and are therefore unable to express a definite opinion on his results. Here again we believe it very probable that the parasite of Delhi boil may eventually prove to be quite distinct from the parasite of Algerian Oriental Sore. Unfortunately nothing is yet known as to how these parasites are transmitted from man to man; as the diseases they produce are benign they offer an excellent opportunity for this line of investigation; without doubt the solution of this problem would help to a better understanding of the methods by which the more dangerous parasites are transmitted.

Quite recently Schneider (1909) has brought forward an interesting observation, which suggests that the parasite of Persian Oriental Sore may be transmitted by the bed bug (species not named, but almost certainly *C. rotundatus*):

A European lady, who lived for some time at Zerguendeh, a village close to Teheran, stated that she awaked one night with the sensation of something biting her on the cheek; putting up her hand to feel what it was, she caught a bug. A little later a large sore developed on the spot where the bug had bitten her. Schneider states that in the same house there were four children, who had the sore the preceding year, and that the house was infested with bugs. Schneider however does not state that the characteristic parasite was recovered from the sores.

Laveran (1909) and others have suggested that the domestic fly is the most probable transmitter of this parasite because the sores usually occur on uncovered parts such as the face, hands and arms. This however is most improbable for the simple reason that it would be impossible for the fly to obtain the parasites, as they do not occur at the surfaces of these sores; it is well known that these parasites cannot live in the presence of septic organisms. *H. tropica* can only be transmitted by a blood-sucking insect which must obtain it from the peripheral blood. It is not true that bed bugs only bite covered parts of the body, for from a large experience of the habits of this insect we know that it chiefly bites uncovered parts of the body. We have visited a large number of native houses in Madras, and found that the majority of the occupants sleep on the floor, either in a room or in rows along the verandahs. The parts of their bodies that are uncovered are their legs, arms, head and neck and often the whole chest if the weather is very warm. In every house *C. rotundatus* will be found in large numbers in crevices in the walls, doors, chairs, beds and mats; and during the night they come out and attack the uncovered parts of the sleepers, biting chiefly round the neck, also the face, hands, feet and ankles. We have no doubt that these conditions

hold good for other tropical countries. As a result of our experience we find bugs are rarely carried about in people's clothes except perhaps by those who are exceptionally dirty and never change their clothes, such as Tibetans and Chinese; in these cases bugs might be able to breed in the folds of their clothing. We have found that *C. rotundatus* prefers to breed and lay its eggs in places where it is likely to remain undisturbed, such as crevices in walls, doors, beds, tables and chairs. Knowing these facts then we can see no reason why the bed bug may not be a suitable transmitter of Oriental Sore.

In appendix B, Woodcock deals with the Spirochaetes, and after referring to Schaudinn's views regarding these parasites, dogmatically asserts that, "the preponderance of opinion is decidedly against these organisms belonging to the Protozoa." No references are given in support of this assertion, and we have no hesitation in saying it is inaccurate.

Woodcock makes no mention of *Spirochaeta duttoni* although it is a blood parasite, and together with *S. carteri* is the best known of the disease-producing Spirochaetes. Woodcock is apparently unaware of Breinl and Kinghorn's (1906) experimental study of the parasite of African Tick Fever; also Dutton and Todd's (1907) note on the morphology of the same parasite, or else he chooses to ignore these papers. The morphological and biological characters of spirochaetes, which determine their inclusion in the Protozoa, have been summarised by Nuttall (1908) in a recent paper, which Woodcock has also ignored. No reference is made to the other important blood-inhabiting spirochaetes, such as *S. anserina, S. theileri* and *S. marchouxi* and many others. Some of these, for instance, *S. duttoni, S. theileri* and certain leech spirochaetes, are known to be transmitted hereditarily; these, together with other well-established facts, leave no doubt that spirochaetes are true Protozoa.

Woodcock then deals in an unsatisfactory manner with "*Trypanosoma*" *balbianii* found by Certes (1882) in the crystalline style and digestive tract of the oyster, stating that this organism "greatly resembles (in structure) a true Spirochaete." No reference is given to Fantham's (1907—08) important work on this spirochaete, and it is not a fair statement "that Perrin's (1906) whole paper thereon is most unconvincing." Woodcock is hardly in a position to make this emphatic statement, unless he has personally worked for some considerable time at these difficult parasites, and we are not aware that he has published any observations on Spirochaetes. If he had studied these parasites, we feel

sure that he would not have stated that "*T.*" *balbianii* more than resembles a Spirochaete, for it actually *is* a Spirochaete, and should now be called *Spirochaeta balbianii* (see Fantham, 1907—08).

Treponema pallidum, which was found by Schaudinn in syphilitic lesions, is next discussed, but in a most incomplete manner. It is obvious Woodcock has not verified his references, for he states that Krzysztalowicz and Siedlecki "in a *recent* memoir" (the italics are ours) have given a detailed account of this organism; the paper referred to was written in 1905 (see the Author's References to Literature, p. 273). No mention is made of Krzysztalowicz and Siedlecki's later paper (March 1908) on *Treponema* (*Spirochaeta*) *pallidum*, in which they state that the Spirochaetes should be placed in a distinct section of the *Mastigophora* for which they propose the name *Spirilloflagellata*. We do not entirely agree with the formation of such a group, but think the omission of any reference to this important paper is unfortunate.

In dealing with the Spirochaetes, we note that Woodcock does not refer to any paper published since 1906, and most of those quoted by him are not later than 1905. It is unnecessary therefore to deal any further with his dogmatic assertion that the Spirochaetes are Bacteria. We are sorry to note the entire absence of figures of Spirochaetes, and that these organisms are less satisfactorily treated here than in his (1906) earlier article on the Haemoflagellates. It is a great pity that in a memoir, which claims to be comprehensive, the Spirochaetes, which are closely allied to the Haemoflagellates, are dismissed in a little more than a page, and are then only noted from a standpoint which is biassed and out of date. Woodcock has missed a great opportunity of giving a broad yet succinct account of the Spirochaetes in this English treatise of Zoology.

In the postscript Woodcock refers to Roubaud's (1908) recent work on certain peculiar developmental forms of lethal trypanosomes in the proboscis of *G. palpalis*. We (1909) have recently criticised this work and pointed out that there is no evidence to show that the flagellates seen by Roubaud in the proboscis of *G. palpalis* are anything more than natural parasites of the fly. We have suggested that the various forms seen by Roubaud in this tsetse fly may quite well represent two distinct species; one of these may be *C. grayi*, and the other *C. tullochi*. Roubaud, as well as all the other observers who have studied these flagellates of tsetse flies, have failed to inoculate animals with them; surely this fact proves conclusively that these parasites have nothing whatever to do with any lethal trypanosome.

Woodcock has compiled a very complete list of all the known hosts of the haemoflagellates and allied forms; this list ought to be of great use to subsequent observers. The flagellates of insects and leeches are confused with trypanosomes, and we note that the *Crithidia* we described from *Gerris fossarum* is wrongly called a *Herpetomonas*. A fairly complete list of references to the literature concludes the article; many recent important papers are however entirely omitted.

After having carefully read Woodcock's article on the Haemoflagellates we regret we are unable to recommend it to students of Tropical Medicine and Protozoology as it is already out of date and quite inadequate for their requirements; it is too full of hypotheses and dogmatic assertions, which are based, in many instances, on erroneous observations. It is quite evident Woodcock has had very little practical experience with the majority of the parasites he describes, his facts having been collected from various sources and containing nothing original. The result is that he is not in a position to form a sound judgment on the mass of observations he has attempted to describe. Under the circumstances we consider Woodcock would have done much better had he contented himself with a detailed description of the various Haemoflagellates and the numerous insect forms, adding to this some original drawings.

Before concluding our review, we wish to indicate what we consider should be the future lines of research on these important parasites. It is now conclusively proved that certain trypanosomes, *T. gambiense, T. brucei* and *T. lewisi* are transmitted mechanically by insects, for instance *T. gambiense* by *G. palpalis, T. brucei* by *G. morsitans, G. pallidipes* and *G. palpalis.*

Excluding the natural flagellates of these insects, there is no evidence to show that these trypanosomes undergo a true developmental cycle in the alimentary tracts of their insect transmitters. Where then do these trypanosomes pass the important stages of their life histories? Salvin-Moore and Breinl's recent observations suggest that some of these blood flagellates manifest a cyclical metamorphosis, which corresponds closely to an alternate absence and presence of the parasites in the blood of their vertebrate hosts. Latent bodies consisting of a nucleus and a vesicle surrounded by a delicate covering of cytoplasm are formed when the parasites are at their minimum in the peripheral blood. These non-flagellate stages are found in the spleen and bone-marrow, and later give rise to flagellates. During the formation of these latent bodies there is an interaction between the blepharoplast and the nucleus; does this represent a sexual phase? In the light of our knowledge that a number

of these trypanosomes undergo no true cyclical changes in their transmitting insects, it is natural to infer that this cycle takes place in the blood and organs of their vertebrate hosts. We would therefore like to see this side of the problem carefully investigated and to know whether Salvin-Moore and Breinl are correct in their conclusions[1].

We would then suggest that future research on the trypanosomes and allied forms be conducted along the following lines:

1. Careful transmission experiments should be carried out with the various blood-sucking arthropods and leeches, in order to ascertain which particular invertebrate is capable of transmitting a given trypanosome or trypanoplasm, and for how long after it has fed on infected blood.

2. Having found the right invertebrate, an exhaustive study should be made of its natural flagellates, devoting particular attention to the methods of transmission of these parasites; they may be transmitted by means of cysts or by true hereditary infection. Having acquired this knowledge, it would then be possible to exclude any natural flagellate, and to study the possible evolutionary cycle of the vertebrate trypanosome, in the digestive tract of the transmitting invertebrate.

3. Should it eventually be found that the trypanosome does not pass through a cycle of development in the invertebrate host, a careful search should be made for a cyclical metamorphosis in the blood and organs of the vertebrate host.

Concluding Remarks.

Our object, in writing this review, is to show that, as there is at present endless confusion regarding such important problems as the life cycles of trypanosomes and the methods by which they are transmitted, it is quite impossible for anyone who has not made a special study of these parasites to attempt to analyse the great mass of conflicting statements, and to differentiate between those that are erroneous and those that are correct.

As we have devoted particular attention to these parasites, more especially to those harmless forms which are parasitic in the alimentary tracts of arthropods and leeches, we have felt justified in criticising the conclusions arrived at by Woodcock and others, pointing out why we consider their deductions are faulty. The preparation of this paper has entailed a great deal of labour, owing to the fact that the observations

[1] Some confirmation of these observations has recently been brought forward by Chagas (1909).

already recorded are scattered in many journals, a few of which we have not been able to consult: further, in many instances the conclusions of one observer are totally different to those of another, although presumably both were studying the same parasite. The observations on the flagellates of insects recorded by most observers are so incomplete and disconnected that, although we have made a special study of the life cycles of several species of *Herpetomonas* and *Crithidia*, it has been quite impossible to place all the observations in any systematic order and to harmonise them with our results. We (1908) have elsewhere suggested that these simple parasites have a definite life history which may conveniently be divided into three stages, *pre-flagellate*, *flagellate* and *post-flagellate*. We believe, that if they are studied along these lines, the many forms, which at present it is impossible to understand, will eventually be found to fit into their right places in the life cycles of the parasites. One example here will suffice. We have already noted that Chatton and Alilaire (1908) have recently described two flagellates from *Drosophila confusa*; one of these is obviously a typical *Herpetomonas*, and the other, which was chiefly found in the malpighian tubes of the fly, we believe is a *Crithidia*. Chatton and Alilaire however consider it is a trypanosome somewhat like *T. dimorphon*, Dutton and Todd. On referring to the authors' figures of this flagellate we find it has all the characteristics of an insectan *Crithidia* such as we (1909) have recently defined; its large blepharoplast should be particularly noted. In fig. 8 we note the flagellum does not appear to pass right up to the blepharoplast, which is almost at the posterior end. This figure should be compared with our drawings of the post-flagellate stage of *C. ctenophthalmi* where a very similar appearance will be seen in the case of the flea crithidia. We believe then that the two flagellates of *Drosophila confusa* are quite distinct, and that one is *Herpetomonas*, while the other is a *Crithidia*. It is well known that both *Herpetomonas* and *Crithidia* may be found in the same insect. From a study of some of the figures of *C. drosophila* we believe Chatton and Alilaire are dealing with the post-flagellate stage of the parasite. Figs. 4, 5 and 6 depict a typical *Crithidia* in which the blepharoplast tends rather to lie behind the nucleus (see our figure of *C. haemaphysalidis*). In studying these parasites of insects it is of the utmost importance to first ascertain how they are transmitted from one insect to another; having found this out the complete life cycle will in most cases be readily understood.

In conclusion we wish to state that we have found the *Bulletin of the Sleeping Sickness Bureau*, kindly sent to us by the Director of the Bureau, of great help. This monthly publication is valuable as it contains accurate summaries of all the papers dealing with Sleeping Sickness and the flagellates of tsetse flies.

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APPENDIX.

Since the above review was written three papers dealing with trypanosomes and allied flagellates have been published which it will be necessary to refer to shortly here. Bruce and his collaborators (1909) have repeated Kleine's experiments (see p. 107) with *T. gambiense* and *G. palpalis*, and have found that this fly is infective after 16, 19, and 22 days subsequent to its ingesting the trypanosome. This discovery only goes to show that during this period *T. gambiense* remains alive and retains its virulence while in the fly, but it by no means proves it undergoes a developmental cycle. There is at present too great a tendency to conclude that certain trypanosomes must pass through developmental cycles in tsetse flies, for we find that in a leading article on Bruce's recent work in the *British Medical Journal* (May 15th, 1909, page 1200) it is stated, "the fact that trypanosomes develop in tsetse flies may now be said to be established." We are not told what is meant by the term "develop," presumably the writer means a sexual cycle; as far as we are aware there is no proof that trypanosomes pass through any such cycle. To say then that *T. gambiense* has a developmental cycle in *G. palpalis* is pure assumption and the loose usage of the terms "develop" and "developmental cycle" are misleading and are to be strongly deprecated.

We will now consider the possible explanation of the long period of non-infectivity of tsetse flies: (1) From our knowledge of the life-cycles of *Herpetomonas* and *Crithidia* it is conceivable that *T. gambiense*, in addition to the well-known flagellate stage, is ingested by *G. palpalis* in a non-flagellate form, as has been described by Salvin-Moore and Breinl, and that this stage of the parasite becomes a flagellate during the period of non-infectivity; it is however not known whether these forms occur in the peripheral blood of sleeping sickness cases. Whether this is so or not, it would quite well explain the long period of non-infectivity of the flies. (2) The parasites after being ingested by the fly undergo a sexual cycle, perhaps after Schaudinn's plan for *T. noctuae*, and then make their way up to the proboscis of the fly. We have however shown above that this cycle is most improbable. (3) The trypanosomes, after remaining from 14 to 16 days in the stomach of the fly, make their way to its proboscis without passing through any sexual cycle; during this period they may undergo equal and unequal longitudinal division. These seem to

us to be the probable solutions of Kleine and Bruce's work, and at present we are inclined to consider the last explanation as the most likely one.

It can be readily understood that any multiplication forms of *T. gambiense* may be quite easily missed, especially when we consider that *G. palpalis* is nearly always infected with *C. grayi* and *C. tullochi*. This would at once explain why previous observers have failed to find *T. gambiense* in this fly several days after it has fed on infected blood. The multiplication forms of *T. gambiense* are not even known and the close similarity of the crithidia to trypanosomes would obviously lead to great confusion unless the life cycles of these natural flagellates are first carefully worked out.

Up till the present it has been generally believed that *T. gambiense* is transmitted mechanically, that is to say the trypanosomes which remain in the fly's proboscis are inoculated into a fresh host just as if they had been injected with a hypodermic needle. This method of infection must however be the exception, as the majority of the trypanosomes are undoubtedly taken into the stomach of the fly, and some time must necessarily elapse before they can find their way back again to its proboscis; there is no evidence to show that during this period the trypanosomes pass through a sexual cycle. We would again point out that *G. palpalis* is known to be frequently infected with two natural flagellates, and that unless their life-cycles are completely worked out it will be impossible, even with bred flies, to study the changes *T. gambiense* undergoes in *G. palpalis*.

The next paper is a preliminary communication by Chagas (1909) who describes two new trypanosomes, one *T. minasense* from a monkey *Hapale penicillata*, and the other *T. cruzi* from a bug (*Conorrhinus*). The non-flagellate stages of *T. minasense* are found in the lungs of infected monkeys; each parasite, according to Chagas, contains eight small organisms each provided with a bilobed nucleus; on their separating they penetrate the red blood corpuscles and become typical trypanosomes. Chagas considers this represents the process of schizogony. Six to eight hours after a bug (*Conorrhinus*) has fed on an infected monkey the trypanosomes (*T. cruzi*?) in its stomach lose their undulating membrane and flagellum; the indifferent forms then multiply and cysts appear which Chagas considers represent a form of sexual development. In the proctodaeum of the bug crithidia-like forms were found in large numbers. Adult parasites resembling full grown trypanosomes were seen in the body cavity of the bug. It is not quite clear as to whether the

author considers the flagellates found in the species of *Conorrhinus* are developmental forms of *T. minasense* or stages of some other vertebrate trypanosome. We know of a species of *Conorrhinus* (*C. rubrofasciatus*) in Madras in which Donovan (1909) has found a true crithidia. It is therefore most probable that *T. cruzi* is also a natural crithidia of the bug and it would be important to know how it is transmitted from one bug to another. There is no proof in Chagas' preliminary note to show that *T. cruzi* is a vertebrate trypanosome.

The last paper we wish to refer to is by Swingle (1909); this author describes in some detail the life-cycle of *Crithidia melophagia* Flu.; he has come to the conclusion that this flagellate has nothing to do with the sheep, but is a true parasite of *Melophagus ovinus.* We entirely agree with this view and consider it is the only possible explanation of its occurrence in the alimentary tract of the sheep "ked." Swingle brings forward some sound evidence to show that this crithidia is most probably transmitted through the eggs of the insect, as he has been able to find its post-flagellate stages in sections of the ovaries. There appears however to be an attempt at true cyst formation such as we have described for *Crithidia gerridis* and *Crithidia tabani.*

With regard to the biological position of this parasite Swingle considers it is intermediate between crithidia and herpetomonas. We however have no doubt that it is a true crithidia, and exactly answers to our (1909) definition of this genus. Swingle rightly points out that if Novy, MacNeal, and Torrey's (1907) nomenclature is to be followed *Crithidia melophagia* would be neither a crithidia nor a herpetomonas, for according to the American observers herpetomonas has an undulating membrane. This we cannot agree with; all the herpetomonads we have studied have no undulating membrane, while all the crithidia have. As we have pointed out elsewhere, the American observers' characterisation of the crithidia is based on Léger's unfortunate description of *C. fasciculata.* Donovan (1909), who has studied these parasites under the most favourable conditions, viz. in the alimentary tracts of their hosts, is entirely of our opinion, and we cannot do better than quote his remarks. He says: "There appears to be still a considerable amount of confusion, one may say ignorance, of the three parasitic genera, Herpetomonas, Crithidia and Trypanosoma; all forms not in possession of an undulatory membrane and a posteriorly placed blepharoplast are loosely called 'herpetomonas forms' or 'wild trypanosomes' (*sic*). As I have had under observation during the last 18 months a number of these three genera I may be pardoned for my presumption in setting this question on a sounder basis.

The full grown form of the genus *Herpetomonas* is characterised by a blepharoplast placed almost at the very extreme anterior end and with no signs of an undulatory membrane. The largest forms are found in this genus, for instance, *H. muscae-domesticae* 70 to 120 μ, including the flagellum. This genus occurs in the non-biting flies and plant-frequenting bugs. In *Crithidia* the blepharoplast is located near and anterior to the nucleus; there is also present what may be called a rudimentary undulatory membrane. The parasites of this genus are found in the gut of the biting flies, in predatory and plant-frequenting bugs, in mosquitoes and in ticks. In *Trypanosoma* we have the blepharoplast placed at the very extreme posterior end and an undulatory membrane very well developed. These forms are parasitic in the vertebrates." Donovan agrees with us that the type species of the genus *Herpetomonas* "is not biflagellate as Prowazek has attempted to make out and such individuals are by far the most common kind met with in the gut of some *Musca domestica*."

THE HAEMOGREGARINES OF MAMMALS AND REPTILES; A REJOINDER TO DR SAMBON.

By CAPTAIN W. S. PATTON, I.M.S.

In a recent paper Dr Sambon (1909) has made a violent attack on me for my gentle reminder to him that he has overstepped the boundary of established truths regarding the haemogregarines, and has let his imagination run wild. As he still persists in maintaining his position I feel I am bound to expose his methods, and in the present paper I propose examining in some detail his recent observations on the haemogregarines, and at the same time dealing with his reply to my paper.

I have read all of Dr Sambon's (1908—9) papers on the haemogregarines carefully, and as far as I can gather his knowledge of them is chiefly based on the work of others, much of which he has wrongly interpreted as I shall point out later. He has in addition studied some haemogregarines from the following snakes:

Python molurus	one	specimen	infected
P. spilotes	„	„	„
Boa constrictor	„	„	„
Corallus cookii	„	„	„
Eryx conicus	„	„	„
Tropidonotus fasciatus	„	„	„
Pseudaspis cana	„	„	„
Zamenis flagelliformis	„	„	„
Coluber corais	„	„	„
C. melanoleucus	„	„	„
Coronella getula	„	„	„
Psammophis sibilans	„	„	„
Naia tripudians	„	„	„
Lachesis lanceolatus	„	„	„
L. mutus	„	„	„

Ten of the haemogregarines found in the above snakes have been made new species. Dr Sambon however tells us very little about the methods he employed when studying these haemogregarines; the snakes were examined at the Prosectorium of the Zoological Society's Gardens, and were dead, I suppose, at the time Dr Sambon made his discoveries. I presume therefore, that as Dr Sambon considers himself an authority on these parasites, he does not think it necessary to tell his readers about the methods which have led to his discovery of the sexual cycle of the haemogregarines.

In his classification of the Haemoprotozoa he tells us the haemogregarine oökinete encysts and produces sporozoites in secondary cysts or sporebags. I am quite at a loss to understand this very misleading statement, unless it is that Dr Sambon is anxious to be the first to predict the probable method of sexual reproduction of the haemogregarines. Judging from a recent leading article in the *Journal of Tropical Medicine and Hygiene*, Dr Sambon is said to have the gift of prophecy or the rare talent of drawing reasonable inferences from the analogy of established truths. Yet I hope to show, that his inferences regarding the life cycles of the haemogregarines are, at present at any rate, premature. Every Protozoologist now knows, that the great interest attached to these parasites lies in the discovery of their complete life cycles, yet there is not at present a single convincing description of such a cycle. The mere recording of new species is now of little interest, as these parasites are so numerous, that anyone who looks with some degree of care in the blood of mammals and reptiles can hardly fail to discover them.

In Dr Sambon's opening paragraph on the haemogregarines of snakes he states that he proposes "to gather all the scattered information concerning haemogregarines in general." Had he only limited himself to this, and recorded the parasites he found in snakes, no one could have taken any exception to his statements, but when he proceeds to interpret the observations of others it is obvious he is not in a position to do so. Speaking of *Lankesterella minima* he says, "in 1871 Ray Lankester also noticed and figured the sporonts of *H. minima*." I would like to ask how Dr Sambon knows that the free vermicules of *L. minima* as figured by Lankester (1871) represent the sporonts of the parasite? By this term I understand that stage of a protozoon which is destined to undergo sporogony, wherever that may take place. Lankester himself says nothing to lead me to think he considered the free vermicules were sporonts. Surely Dr Sambon does not expect his readers to accept this

interpretation of Lankester's observations without convincing evidence to prove it; further I can find nothing in Dr Sambon's papers to show that he has even studied this parasite. These however are just his methods, and throughout his papers he interprets other workers' observations to suit his own ideas. The principle seems to be, Dr Sambon makes the free vermicules of haemogregarines sporonts, therefore they must be sporonts.

As I have studied *L. minima* I am in a position to examine any evidence in support of Dr Sambon's view. It is well known that on examining, in the fresh condition, the blood of a frog infected with this haemogregarine, the parasites soon leave the red blood corpuscles and may be seen as free vermicules moving about in the plasma. On feeding a species of *Glossiphonia* parasitic on *Rana tigrina*, and on examining its crop contents, it was found that the parasites had similarly liberated themselves, and were actively moving about in the fluid. Now if these parasites represent the sporonts it is only natural to expect that they would, after some unknown time, begin the process of sporogony somewhere in the leech's alimentary tract, and according to Dr Sambon we should expect to find them in couples lying side by side. Later they would have fused together and then produced an oökinete, which would eventually result in a cyst containing sporozoites. Now nothing of the sort takes place, the free vermicules (Dr Sambon's sporonts) do not undergo any such process, for they can be found in much the same condition in the crop of the leech for several days after it has sucked the blood of an infected frog. What then happens to them and how does the parasite complete its evolution? From some evidence I have been able to gather I believe the parasites make their way to the sheath of the proboscis, and are then inoculated into the next frog the leech bites. This is the only conclusion I can come to at present, and though it may seem strange to Dr Sambon that this parasite does not undergo sporogony in its invertebrate host, no amount of observations on the vermicules in the leech can demonstrate such a cycle. I suppose Dr Sambon would tell me, that had he examined the vermicules of *L. minima*, he would have been able to say whether in this case the sporonts exhibit any sexual differentiation, for he now tells me, that as he has not seen the leucocytic parasite of the hare, he is not in a position to say whether its sporonts do at any time exhibit sexual differentiation. I begin to wonder now whether there has been something wrong with the methods and technique I have employed in studying these parasites, because I am unable to find their sporonts. It is therefore to be regretted that Dr

Sambon does not give us at least some hints as to how he has been so fortunate in finding them, especially those of *L. minima*, even though he appears not to have studied this parasite.

When discussing the life history of the haemogregarines Dr Sambon begins by saying that, "like that of other haemoprotozoa, (it) is divided into two cycles; a schizogonic or 'vegetative' cycle spent in the blood of vertebrates and characterised by asexual multiplication, and a sporogonic or sexual cycle spent in the digestive organs of blood-sucking invertebrates and characterised by asexual reproduction." When I first read this statement I thought that Dr Sambon had been more fortunate than I have been, but as I read his papers further I discovered that he had not found anything whatever to show that the life history of a haemogregarine like that of other haemoprotozoa is divided into two cycles. As I have so far looked in vain for the sexual cycle of a haemogregarine thinking it would be something like that of the Coccidia, I was disappointed to find that after all Dr Sambon had discovered it was like that of other haemoprotozoa. Exactly what haemoprotozoa he refers to I am not sure of; as far as I am aware the malarial parasite is the only one whose life cycle we know with any certainty. Dr Sambon is evidently sure of the life cycles of some other haemoprotozoa, possibly he refers to the trypanosomes. I need hardly remind him that as far as we know at present these parasites do not pass through the two cycles referred to above.

Under the heading "schizogonic cycle," Dr Sambon says that "in examining *fresh blood* (the italics are mine) from vertebrates harbouring haemogregarines we find these parasites usually enclosed within the blood-cells they select for their development,..... For convenience of description we may distinguish three principal forms: *Young forms*, oval, fusiform, or club-shaped, with nucleus median, large, round or oval, homogeneous. At first they are free within the stroma of their host-cell; later encapsuled. *Adult sporonts*, club-shaped, more or less bulky, usually doubled up, always encapsuled, nucleus median, with chromatin filament forming a more or less open skein, or broken up into rods. Host's cells as a rule unaltered." Here we have a statement that certain of these parasites when seen in the fresh blood with appearances described above are young forms, but how does Dr Sambon know they are young forms? He gives no figures showing that the young merozoits just liberated, say, from a cyst in the lung of a snake, are exactly similar to the parasites he describes as young forms. Yet this description is apparently meant to hold good for all haemogre-

garines. As he has not studied the eomplete eyele of a haemogregarine in the blood and organs of his snakes, I fail to see how he is in a position to make the statements I have quoted above. In criticising my remarks on his schizonts and sporonts he says: "After declaring that he cannot reeognise any specifie differenee between the haemogregarines from different genera and families of snakes, Captain Patton goes on to admit that he is unable to recognise any difference of stage in the development of any of the many examples examined." This is a misstatement and elearly exemplifies Dr Sambon's method of controversy. I have nowhere said I eould not recognise any differenees in the stages of development of snake or other haemogregarines, whether they be from the peripheral blood or from the organs of their hosts. If Dr Sambon will only read my paper carefully he will find it stated, that I have examined many examples of all these stages, but that I am not in a position at present to interpret them as young forms, adult sporonts, and adult sehizonts; this is what I admitted, a very different thing to what Dr Sambon tries to infer. I also said that, "without infecting a snake through the ageney of the right tick, and then studying the various forms of the parasites that appear in the blood and organs of the snake, I do not see how it is possible to speak of the parasites in the peripheral blood as sehizonts, sporonts, ete." Dr Sambon has earefully avoided this sentenee, and ridieules the previous one by saying that he fails to understand the significanee of the presenee or absenee of tieks on snakes; he reminds me that we do not diagnose the species of malarial parasite by determining the kind of mosquito which ingests the parasite. This I should think is quite obvious to anyone; as Dr Sambon is not in a position to appreeiate my remarks on ticks and snake haemogregarines I will explain it further.

The method of multiplieation of the haemogregarines of snakes in their lungs and livers is a complieated proeess, two distinct eysts being formed whieh eontain two forms of the parasites. On examining infected snakes, I have found that both these cysts are almost invariably present, and that it is quite impossible to say whether one or both represent sehizogony, or a modified form of sporogony; at present I am inelined to the latter view. If it is impossible to express a definite opinion on the nature of these eysts and their contents, it is in my opinion equally impossible to say what stages the parasites in the peripheral blood represent. In order therefore to overeome this diffieulty another method of studying these parasites must be adopted. A number of infeeted snakes harbouring tieks should first be colleeted;

in Madras I find the rat snake, *Zamenis mucosus*, is well adapted for this purpose. It is then necessary to obtain some uninfected snakes of the same species, and by careful feeding experiments with the ticks to transmit the parasites. Having ascertained the method of infection as well as the approximate time the parasites take to appear in the peripheral blood of the snakes, it is necessary to repeat the experiment, and after a short interval to examine the lungs of the snakes for the process of multiplication of the parasites. It will then be possible to see what type of cyst first appears in their lungs and livers, and what form of parasite is at the same time discharged into the general circulation. This is the plan I have adopted in studying these parasites of mammals and reptiles, and I believe it is the only way their complete life cycles can be worked out. It can be readily understood that it is very tedious work, requiring great patience as numerous initial difficulties have to be overcome.

Perhaps now Dr Sambon will understand my reason for referring to the presence or absence of ticks on snakes, and also why I consider it is impossible at present to say definitely, that certain parasites in the peripheral blood of snakes infected with haemogregarines represent schizonts, sporonts, and so on. Dr Sambon however believes that he is able to recognise these various stages, and mentions the well known forms of the malarial parasite in the peripheral blood of patients infected with this parasite, a truth I am well acquainted with; but have the haemogregarines analogous stages? Dr Sambon assumes they have, not a very remarkable discovery in itself, but an assumption at present quite unjustifiable to say the least of it. Dr Sambon, I note, also states, "that the multiplication forms in the lungs of snakes do belong to the schizogonic cycle there can be no doubt. The adult schizonts of snake haemogregarines as far as we know invariably and exclusively break up within the lungs of their hosts." The multiplication forms of snake haemogregarines do not exclusively develop in the lungs of snakes, but are just as common in their livers; Dr Sambon apparently is not aware of this fact. He need therefore hardly remind me, that these stages of snake haemogregarines in their vertebrate hosts are well known, and have been described by Lutz, Wenyon and himself[1]. Although Dr Sambon speaks of the schizogony of all his snake haemogregarines as occurring in the erythrocytes of the snakes, I can find no description of this cycle; he appears however to have seen the cysts of "*H. seligmanni*"

[1] To be accurate it is necessary to note, that Dr Sambon's description has yet to be published.

after he had written his papers. Dr Sambon then goes on to adopt his usual method of bringing in everything he knows that may have even the remotest bearing on the subject, regardless of the fact that most of what he says has nothing to do with the question I raised. What I have said is quite clear, and is in no way connected with the multiplication forms of other haemogregarines, yet Dr Sambon infers that I doubt whether other haemogregarines have such stages, and adds a list of names of observers, two of whom, as far as I know, have not described any of the multiplication forms of these parasites. I need hardly say that I have studied these stages of most of the haemogregarines.

Now with regard to the vexed question of the sexual cycle of haemogregarines, Dr Sambon says under the heading "conjugation," "I can fully confirm Labbé's observations, having witnessed it not only *in vitro* but also in blood taken from the gut of a tick fed on an haemogregarine-infected lizard"; nothing further is said about this parasite, which I presume is *H. ehrlichi*. Dr Sambon continues, "I have had the opportunity of examining the process of accouplement so frequently in *Haemogregarina seligmanni* that I have no doubt whatever about it." On the same page there are three figures which are not named, and which are not even referred to in the text, I can only presume therefore that they represent the sporonts of *H. seligmanni* in accouplement.

They appear to have proceeded only a little way towards conjugation, and Dr Sambon himself says, "I have not seen the nuclei of the conjugating haemogregarines unite." Yet he thinks this must take place in the gut of the invertebrate host, in this case I suppose in *Porocephalus crotali*. After having brought the reader breathlessly up to this point, eagerly expecting to have the mystery of the sexual cycle of these haemogregarines solved, Dr Sambon suddenly digresses to describe such uninteresting points as the structure and motility of the free sporonts. There is no proof that the parasites he figures are actually undergoing the process of conjugation, and I have certainly never seen any such process in snake haemogregarines, either *in vitro*, or in the alimentary tracts of ticks and linguatulids. Nothing more is said about the conjugation and further stages in the sporogony of "*H. seligmanni*"; Dr Sambon however says in a footnote that they will be described in a future paper. Under the heading "sporogony," Dr Sambon merely re-describes the observations of Simond (1904), Durham (1902), Billet (1904), Brumpt (1904), Christophers (1905), Laveran and Nègre (1905), and Prowazek (1908). With these conflicting statements and his limited observations on snake haemogregarines, Dr Sambon does

not hesitate to predict the method of sexual reproduction of all the haemogregarines.

When criticising my remarks on *L. leporis* in the tick *H. flava* Dr Sambon refers to Miller's (1909) recent paper on *Leucocytozoon* (*Hepatozoon*) *perniciosum*, and states that Miller has proved, "that the sporogony of this species occurs in a rat mite, *Lelaps echidninus*," and that Miller's investigations have "proved the correctness of the account I gave of the haemogregarines in my classification of the Haemoprotozoa which Sir Patrick Manson did me the honour of adopting and supporting in the fourth edition of his *Manual of Tropical Diseases*." Here we have it that in 1907 Dr Sambon prophetically predicted the probable method of sporogony of the haemogregarines, and that now Miller's work—though as yet unconfirmed—has proved the accuracy of Dr Sambon's prediction. This is however regardless of the fact that at the time Dr Sambon classified the haemoprotozoa, he had in my opinion no grounds whatever for saying that the haemogregarine oökinete encysts and produces sporozoites in secondary cysts or sporebags. This, I suppose, is what Dr Sambon calls "dabbling in zoological matters."

Miller summarises the sexual cycle of *L. perniciosum* as follows: "When the blood of an infected rat is swallowed by a mite the encysted trophozoits are set free in the stomach by solution of the cyst as free vermicules. Two similar vermicules become associated and conjugate. One, the macrogamete, grows larger and partly surrounds the other, the microgamete. The protoplasm becomes fused and later the nuclei conjugate and fuse to form a zygote. The zygote becomes a sluggishly motile oökinet, which penetrates the stomach wall of the mite and enters the body tissues and becomes encysted (oöcyst). Here a remarkable enlargement of the karyosome takes place. The parasite increases enormously in size. The nucleus of the spherical sporont thus formed undergoes division into many daughter nuclei, which migrate to the surface of the sporont. The surface of the latter becomes mammillated. The projections, each of which contains a nucleus, increase in size and length; later they are broken off and each becomes a sporoblast. The nucleus of the sporoblast undergoes division, the resulting nuclei being arranged at the poles. The sporoblast increases in size and a cyst wall developes. Around each nucleus a sporozoite is formed. In the ripe sporocyst, which measures 25 by 30 micra, the sporozoites, 16 in number (average) are arranged at the poles. The large cyst (oöcyst) contains from 50 to 100 of such sporocysts. When the mite is swallowed by a rat the cycle is repeated."

This account of the sexual cycle of the parasite at once recalls the appearances I have seen in *Porocephalus pattoni* from the lung of *Zamenis mucosus*, and are also very similar to those described by Christophers (1905) from the body cavity of *Haematopinus stephensi* from *Gerbillus indicus*. Not only are the ripe cysts in each case almost identical in that they contain small cysts full of sausage-shaped bodies, but the earlier stages I have seen in the linguatulid and those described by Christophers from the louse are very striking in their similarity. I have no doubt therefore that the parasites I have seen in *P. pattoni*, and those described by Christophers from the louse, and by Miller in the mite, represent stages in the development of different species of the same genus of a sporozoon. If we are to accept Miller's work as correct it follows that the parasites (cysts) seen by Prowazek, Sambon and myself in linguatulids represent the various stages in the sexual cycle of the haemogregarines of *Python reticulatus*, *Lachesis mutus* and *Zamenis mucosus*. The question then arises how are the sporozoites of these haemogregarines transmitted from an infected to an uninfected snake? According to Miller's conclusions it would be necessary for the uninfected snakes to swallow the infected linguatulids. It is not definitely known how snakes become infected with these arthropods, but it is believed they swallow their eggs or immature stages in their food; for instance the rat snake, *Zamenis mucosus*, probably swallows the eggs of *P. pattoni* in the frogs and toads, which are its principal food. At any rate it is well established that snakes harbour the adult stages of linguatulids, and are the definitive hosts of these arthropods. It is therefore most improbable that snakes swallow adult linguatulids, and if this is true, it is impossible at present to understand how snakes harbouring linguatulids can become infected with the sporozoites of haemogregarines in these arthropods. If it is impossible to understand how this method of infection can take place, it is equally as difficult to understand how an uninfected snake could become infected by the bite of an adult (haemogregarine-infected) linguatulid. Realising these difficulties in 1905, when I first found the cysts in *P. pattoni*, I came to the conclusion that these parasites did not represent stages in the sexual cycle of a haemogregarine. I have examined over 60 specimens of this linguatulid and could trace no connection between the haemogregarine of *Zamenis mucosus* and the parasites in *P. pattoni*. With regard to the cysts Prowazek (1908) has recently found in *P. moniliformis*, I pointed out that he suggests that they may represent a further development of *H. pythonis*; Dr Sambon however speaks of "Prowazek's discovery of

oökinetes and encapsuled oöcysts of *Haemogregarina pythonis*," as if it were absolutely proved by Prowazek that they represent the sexual stages of *H. pythonis*. Why Dr Sambon exaggerates Prowazek's statements I cannot understand, unless he thinks by doing so he will strengthen his own position. He now states that in consequence of Prowazek's announcement he has examined some of the specimens of *P. crotali* from *Lachesis mutus* infected with *H. seligmanni*, and has been able to confirm Prowazek's discovery, and thus complete the life history of this haemogregarine. He tells me that I am wrong in considering the haemogregarine oökinetes and oöcysts found in the stomach of *Porocephalus pattoni* as stages in the development of a parasite peculiar to the linguatulid. Dr Sambon surely does not think that I would have lost the opportunity of describing the sexual cycle of a snake haemogregarine which I found in 1905, and left it to Prowazek and himself to re-discover and describe. If Dr Sambon will refer to the Annual Report of the Bacteriological Section of the King Institute of Preventive Medicine for 1906 he will find the parasite of *P. pattoni* recorded there provisionally as a Gregarine; at present I see no reason to alter my opinion. I shall however look forward to reading Dr Sambon's description of the complete life cycle of *H. seligmanni*; I only hope that he will give us some definite proofs to support his statements.

Dr Sambon, in criticising my remarks on the developmental forms of haemogregarines in linguatulids, says, "Captain Patton is inclined to consider all the developmental forms of vertebrate haemoprotozoa found within the alimentary tubes of invertebrate hosts as totally independent parasites peculiar to these invertebrate hosts." It is now well known that blood-sucking invertebrates are infected with natural parasites whose life cycles are very imperfectly known, and as there is at present too great a haste to discover and describe the developmental forms (sexual cycles) of blood-inhabiting protozoa in the alimentary tracts of invertebrates, these natural parasites have been entirely overlooked. Recent protozoological literature is full of such mistakes. Knowing these facts it is necessary to observe great caution in interpreting the forms found in the digestive tubes of invertebrates fed on the blood of vertebrates infected with protozoa. This is what I wish to emphasise when I disagree with Dr Sambon in his interpretation of the parasites of linguatulids; I have however nowhere stated that *all* the developmental forms of vertebrate haemoprotozoa found in the alimentary tracts of blood-sucking invertebrates represent stages in the evolution of parasites peculiar to invertebrates. As for Dr Sambon's reference to

the herpetomonas stages of trypanosomes of vertebrates in the alimentary tracts of diptera, I hardly think he is in a position to criticise my work along these lines until he can prove that vertebrate trypanosomes have such stages; perhaps he may then be able to say I am wrong.

As I have so far been unable to find any developmental stages of haemogregarines in invertebrates I can only tentatively accept Miller's conclusions. It remains to be proved whether these blood parasites of mammals and reptiles will eventually be found to be transmitted in the extraordinary way suggested by Miller's recent work. I am certainly not so sanguine about it as Dr Sambon is. A great deal of work has yet to be done before it can be said for certain that the haemogregarine oökinete encysts and produces sporozoites in secondary cysts or sporebags.

I will now give the last example of how Dr Sambon interprets other workers' observations. In speaking of *Leucocytozoon funambuli*, he says, "In 1906, Captain Patton, in describing *Haemogregarina funambuli*, a parasite of the five-striped palm squirrel (*Funambulus pennantii*), stated that he had seen free sporonts in the stomach of the squirrel's louse, also a species of *haematopinus*." If Dr Sambon will refer to my (1906) memoir on this parasite he will find that I have nowhere stated that I had seen the free sporonts of the parasite; Dr Sambon apparently knows more about it than I do. In the last Report of the Bacteriological Section of the King Institute I stated, "I have again failed to find any extra-corporeal cycle in the lice found on *Funambulus pennantii*." Dr Sambon either does not know of this Government publication or else he chooses deliberately to ignore it. How can he then in face of these facts say the free vermicules of *L. funambuli* are the sporonts of the parasite?

In a footnote to his paper, Dr Sambon expresses great surprise at Professor Nuttall permitting the use of the name *Leucocytozoon* in his Journal, and says, "The name *Leucocytozoon* cannot be used for the haemogregarines of mammals whether they be parasites of leucocytes or not. It is the generic name of certain avian parasites discovered first by Danilewsky in 1884 in the blood of owls." Dr Sambon will find my reason for retaining these parasites in this genus in my paper on "Mammalian Leucocytozoa" in the Report of the King Institute for 1907. With Laveran I prefer to place the avian parasites in the genus *Haemamoeba*; the justification for this is fully supported by Mezincescu's (1909) recent work. In conclusion I may say I do not intend wasting my time in further discussing with Dr Sambon the points I have raised, even though he should choose to reply to this paper.

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THE SUCCESSFUL DRUG TREATMENT OF CANINE PIROPLASMOSIS, TOGETHER WITH OBSERVATIONS UPON THE EFFECT OF DRUGS ON *PIROPLASMA CANIS*.

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(1 Text Figure.)

CONTENTS.

In a previous paper, Nuttall and Graham-Smith (x. 1908) reported upon various experiments they had conducted with a view to finding a remedy for Canine piroplasmosis. They found that Quinine bihydrochloride, Tartar emetic, Sodium-methyl-arsenate, Methylene blue and Beta-naphthylamine exerted no curative action upon the disease, and they cited experiments conducted by Gonder (1907) which showed

that atoxyl treatment actually aggravated the symptoms of the malady. Although the prospect of finding a remedy which would exert any influence upon the course of the disease appeared very doubtful, we nevertheless considered it desirable to continue these investigations.

Our experiments were conducted in a similar manner to those detailed by Nuttall and Graham-Smith, and the same strain of *P. canis* was used. Our Cambridge strain has been maintained for a considerable time in the laboratory by inoculation from dog to dog and a long series of passages has in no way influenced its virulence. Animals which have been inoculated subcutaneously with the fresh defibrinated heart blood of a dog suffering from the disease are almost certain to die. From our laboratory experience, mainly with young dogs, we reckon upon a mortality of about 90%. The strain we used came originally from Cape Colony. We do not know the source of the strain used by Gonder, but we consider that the parasites he experimented with must have been much less virulent than ours, since two out of three of his *control* dogs recovered (all his atoxyl-treated dogs died). Our reason for making this statement is to show that we have been dealing with a very deadly disease.

After the dogs had been inoculated with defibrinated or citrated blood, rich in parasites, they were kept under continuous observation, their blood, obtained by pricking an earvein, being periodically submitted to microscopic examination, with a view to detecting the parasites. Drug treatment was usually commenced after the parasites appeared in the blood.

The drugs were prepared in watery solution and in most instances the solutions were injected subcutaneously. In the case of Trypanblau and Trypanrot, the dyes soon become diffused throughout the system as evidenced by the blue or red colouration of the animal's skin and mucous membranes. Well-marked colouration was noticeable after 2—3 hours, and usually attained its maximum intensity after about 9 to 12 hours. Sometimes the drug injections produced local swellings, which generally subsided after a short interval of time; in other cases an abscess formed at the site of injection. The abscesses healed rapidly after they were lanced.

Before describing the success we have obtained by means of Trypanblau and Trypanrot treatment, we may as well mention that treatment with Arsacetin (Sodium-p-acetyl-aminophenylarsinate-acetylarsanilate) and Soamin (Sodium-para-amino-phenylarsonate) was without any effect upon the course of the disease.

We herewith subjoin the protocols of two experiments with these drugs:

Arsacetin Experiment.

Day

1. *Dog:* Inoculated with 5 c.c. of defibrinated heart blood.
5. Parasites appeared.
   Treatment begun: 2 c.c. of 5 % arsacetin dissolved in water injected subcutaneously.
7. Treated as before, dog looking very ill, parasites increasing in numbers; haemoglobinuria.
8. Dog died of piroplasmosis.
   Control dog died on 9th day.
   Result: The treated dog died on 8th day, the control dog died on 9th day.

Soamin Experiment.

Day

1. *Dog:* Inoculated with 5 c.c. of defibrinated heart blood.
5. Parasites appeared.
   Treatment begun: 1·5 c.c. of 1 % soamin dissolved in water injected subcutaneously.
6. Fifteen parasites per field.
7. Treated as before, many parasites in blood.
8. Dog ill.
9. Dog died.
   Control dog died on 8th day.
   Result: The treated dog died on 9th day, the control dog died on 8th day.

Note to Diagram on p. 159.

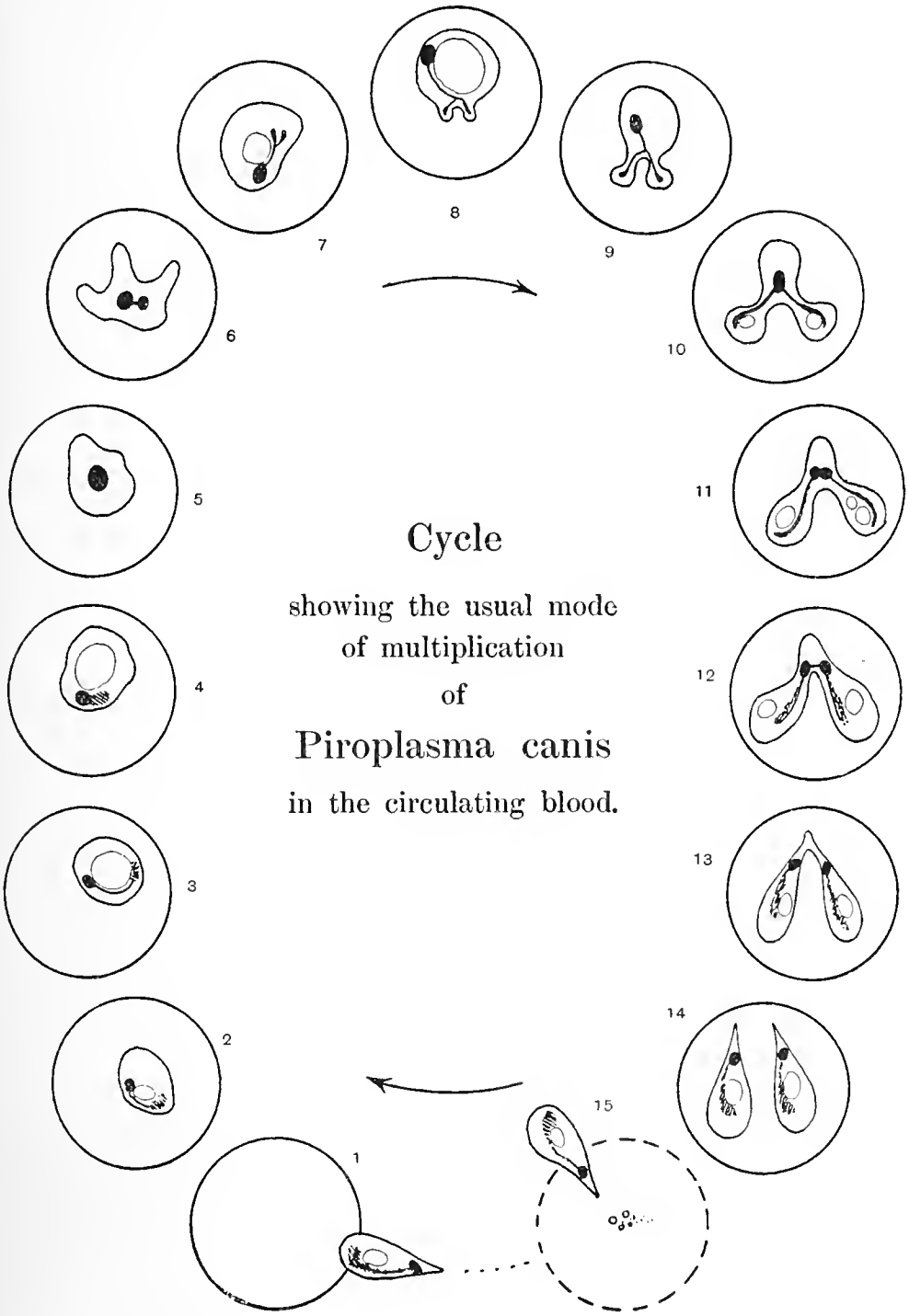
Fig. 1 represents the entrance of a free pyriform parasite into a fresh corpuscle. The parasite retains its pyriform shape for a short time after it has effected an entrance into the corpuscle. The presence of single intracorpuscular pyriform parasites is indicated by the sign (P) in our protocols.

Figs. 2—7 represent successive stages of development: the pyriform parasite becomes rounded, grows in size, becomes amoeboid and undergoes nuclear changes of a peculiar character prior to the protrusion of the bud-like processes, as shown in Fig. 8. Such forms are all grouped under the sign (O) in our protocols.

Figs. 8—12 represent successive stages of development from the first protrusion of the "buds" to the almost completely formed pair of pyriform parasites. Such forms are all grouped as dividing forms under the sign (D) in the protocols. This stage is short-lived.

Figs. 13—14 represent the completely formed pair of pyriform parasites, at first conjoined [13] and then separated [14] from each other. Such forms are grouped under the sign (PP) in the protocols.

Fig. 15 represents the escape of the pyriform parasites from the corpuscle and their almost immediate entry into fresh corpuscles, as seen in Fig. 1.


Cycle
showing the usual mode
of multiplication
of
Piroplasma canis
in the circulating blood.
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Note upon the development of Piroplasma canis in untreated dogs.

Before proceeding to describe the very striking effects exerted by Trypanblau and Trypanrot upon the parasites, it appears expedient to refer briefly to the mode of development of *P. canis*.

For a clearer understanding of the significance of the forms of parasites to which we shall refer in this paper, we reprint a Diagram from Nuttall and Graham-Smith (*Journ. of Hygiene*, 1907, VII. p. 257), wherein is represented the usual mode of multiplication of *Piroplasma canis* in the circulating blood. The figure is based upon extensive studies of the living parasite, supplemented by the examination of stained specimens.

In this Diagram the figures are numbered successively 1 to 15 and represent the stages in the life history of the parasite whose significance is explained in the description of the Diagram (p. 158).

A consideration of the cycle of development described and figured in the Diagram, readily explains the reason for the presence of two prevailing forms of intracorpuscular parasites. Free pyriform parasites are rarely encountered except toward the end of a fatal attack of the disease, when the corpuscles rapidly break down and haemoglobinuria is a prominent and consequent symptom. The reason for there usually being few *free* pyriform parasites is due to the fact that, immediately upon their escape from the corpuscle in which they have developed, they re-enter fresh corpuscles. As a rule, few single pyriform parasites are encountered *within* corpuscles, because after they have penetrated the corpuscle the parasites very soon become rounded. Similarly, the *dividing forms*, which have been overlooked by other observers, are not commonly encountered except in small numbers because the process of division is fairly rapid. At times dividing forms are more plentiful than at others. Some striking figures in this respect can be seen by reference to those of our protocols which include an enumeration of the different forms of parasites encountered in blood films at various stages of the disease.

The two prevailing forms of intracorpuscular parasites are (O), the single rounded or irregular (amoeboid) parasite, and (PP), the pairs of pyriform parasites. These two forms prevail because *they represent phases of development which progress slowly.* That two pairs of pyriform parasites (PPPP) occur more rarely is due to the fact that a rounded parasite usually developes into two pyriform parasites by the process figured in the Diagram; it is a rare occurrence for a

single rounded parasite to give rise directly to four pyriform parasites by the "budding" process. (See Nuttall and Graham-Smith, 1907, p. 240, Diagram XXVII; 1908, p. 137, Diagram II, fig. 21.) The most reasonable explanation of the formation, under ordinary circumstances, of four pyriform parasites (PPPP) in a corpuscle is the same as that which explains the presence of two rounded parasites (OO) in a corpuscle, namely, that the corpuscle was originally invaded by two pyriform parasites which have undergone independent development side by side. It is our belief that the presence of larger numbers (6—12—16) of rounded and pyriform parasites within corpuscles, obtained especially in preparations taken from the internal organs, is due to the slowed circulation in those organs more freely permitting the entry of several pyriform parasites into adjacent corpuscles after they have escaped from the corpuscle in which they have accomplished their development. This does not exclude the occasional occurrence of the direct development of four or possibly more parasites from a large single amoeboid parasite.

The proportion of rounded (O) to pairs of pyriform (PP) parasites varies somewhat in the same dog at different times; thus, in one of our untreated dogs (VIII), the proportion of (O) to (PP) was as 60 to 35 at one time, as 41 to 50 at another, the average proportion obtained from counts made on nine occasions, during a period of three days, being 55 (O) to 39 (PP).

If we calculate the proportion of (PP) to (O) parasites according to their percentage relation to each other, leaving less frequent forms out of account, we find that in control dog VIII for every 100 (O) parasites there were present

	On day 5	Variation within 16 hours on day 6						On day 7 when dog died	
% of (PP) to (O).	58	46	80	60	75	93	122	47	77
% of infected r.b.c.	?	·6	·4	1	2	1·2	2·8	17	28

We shall see that the numerical relation between (O) and (PP) is much disturbed through the administration of the drugs in the experiments we are about to describe.

The method of enumerating the different forms of intracorpuscular parasites as recorded in the protocols.

In consideration of what has been stated in the preceding section and of what is to follow, it will conduce to clearness and brevity to explain the signs used throughout this paper to indicate the different forms of intracorpuscular parasites which are encountered in the blood.

In the following signs, the brackets ( ) represent the corpuscles which include the parasites. Thus:

The sign	stands for a corpuscle harbouring:
(P)	1 pyriform parasite.
(O)	1 usually round, at times irregular or amoeboid, parasite.
(PP)	2 pyriform parasites.
(OO)	2 rounded parasites.
(PPPP)	4 pyriform parasites, usually including types like (PPO), which are rarer, and also the still rarer higher numbers of parasites in a corpuscle.
(PPO)	2 pyriform and one rounded parasite.
(D)	1 dividing form.
(DD)	2 dividing forms.

The abbreviation *r.b.c.* in the protocols stands for red blood corpuscle.

The enumeration of parasites in untreated dogs.

As stated in the previous section, an enumeration of the different forms of parasites soon revealed the fact that certain forms prevail numerically above others. We soon found, that for practical purposes other forms could be safely ignored. Thus, an occasional corpuscle might contain more than four pyriform parasites (PPPP), but these corpuscles were present in very small numbers in the peripheral circulation, and could, in consequence, be included under the heading of "four or more" parasites in a corpuscle. Similarly, two pyriforms and one rounded parasite (PPO) in a corpuscle could usually be included in this category, for we know that the rounded parasites in such a corpuscle may at times multiply by the "budding" process, described by Nuttall and Graham-Smith, and give rise to an additional pair of pyriform parasites. In one case only, that of Dog 10, did the type (PPO) occur in considerable numbers (9 %) in blood films made at autopsy.

When about 200 infected corpuscles are counted and classified according to the forms of parasites which they contain, the forms (P), (D), (OO), (PPPP), may frequently not be encountered. At times they are more numerous. The (O) and (PP) are always present in greater numbers. Thus, by referring to the protocols, it will be seen that parasites of the type

(PP)	may constitute	30—68 %	of the infected corpuscles in untreated dogs at various stages of the disease.
(O)	,, ,,	29—64 %	
(PPPP)	,, ,,	0—20 %	
(D)	,, ,,	0—7 %	
(P)	,, ,,	0—6 %	
(OO)	,, ,,	0—4 %	

It is a significant fact that the highest percentages of parasites belonging under categories (PPPP), (D), (P) and (OO) were counted only in the cases of dogs which died of piroplasmosis. Thus, in Dog 10, a small puppy, there were counted at various times as many as 10 % (PPPP), 7 % (D), 6 % (P) and 4 % (OO) at an early stage of the disease. In Dog 11 there were, on one occasion, as many as 13 % (PPPP) present, whilst in Dog 2 there were no less than 20 % (PPPP) present on the day the animal died. In any case, the percentages above quoted sufficiently prove that the commonest forms of parasites are the (O) and (PP) forms. If the actual number of parasites in a corpuscle were enumerated it naturally follows that the percentage for (PP) parasites would be doubled, for each "P" represents an independent parasite.

In connection with our studies upon the effects of drugs upon *P. canis* we found it necessary to make very careful examinations of the blood both in our control dogs and in those that were treated. This led us to examine the peripheral blood for the earliest appearance of parasites, and, as the disease progressed, to record the percentage of infected to uninfected corpuscles.

At first very few corpuscles are found to harbour parasites, and they can only be found with difficulty along the *edge of the film*, or at the *end* of the film, in the region where the leucocytes are encountered in greatest numbers. At this stage many thousands of uninfected corpuscles occupy the greater part of the film, and it is practically impossible to estimate the percentage of infected corpuscles. We have been satisfied in such cases to note the presence of parasites "only at film-edge." As the disease advances the percentage of infected corpuscles usually rises steadily until it attains its maximum shortly before death (30 to 80 % or more). When the disease runs a more chronic course there may be fluctuations in the percentage of infected to uninfected corpuscles.

The effects of Trypanblau and Trypanrot upon the morphology of Piroplasma canis.

The effect of Trypanblau and Trypanrot upon the morphology of *P. canis* is exceedingly striking. The pyriform parasites rapidly disappear and the proportion of rounded parasites is increased so that after a time all the intracorpuscular parasites appear rounded. Moreover, some of the parasites, when viewed in stained preparations, show visible signs of degeneration in that they may present a ragged or irregular

appearance, whilst masses of chromatin are extruded. Viewed in fresh films, the parasites for the most part appear rounded. After a further interval of time the rounded parasites may disappear from microscopic observation and the animal recovers. In most cases, especially where there has been a heavier infection, the parasites may again be detected microscopically after the lapse of 9 to 12 days, the disease having assumed the chronic type in which few parasites persist in the blood for a considerable period of time.

As soon as we discovered that the drugs exerted a *visible* effect upon the parasites, we determined to make numerical estimations of the different types of parasites encountered in the infected corpuscles of untreated and treated dogs so that comparisons might be made which would give some measure of the effects exerted by the drugs.

Bearing in mind the facts discovered by Nuttall and Graham-Smith regarding the mode of multiplication of *P. canis*, we were very much struck by the fact that the pyriform parasites are so directly affected by the drug. It does not, however, appear difficult to explain their disappearance when we remember that the pyriform parasites, having attained maturity, escape from the corpuscle in which they have developed and pass out into the plasma.

Once in the plasma, and no longer protected by the substance of the corpuscle, they are directly exposed to the effect of the drug. In this respect we have a similar and well-known instance in the effect exerted by quinine upon the escaping merozoites of malarial parasites. In view of the fact that in *P. canis* there is no definite periodicity in respect to the time when the parasites escape from the corpuscles, some hours (3—9) are usually required for cutting short an attack if we still maintain the analogy with the phenomena observed in the treatment of malaria with quinine.

Again, as in malaria, our protocols show that relapses may follow treatment. On what these relapses depend can, in the present state of our knowledge, only be surmised, but we assume that there may be resistant or latent parasites present in the body which develope as soon as the conditions favour their multiplication.

The drugs appear to also exert an influence upon the intracorpuscular parasites, for we have seen evidences of degeneration in the latter. On the other hand we have no direct evidence that the double pyriform intracorpuscular parasites become rounded. If the drug affected the (PP) parasites prior to their escape by causing the pyriform parasites to become rounded, we should expect to find a largely increased per-

centage of (OO) parasites, but such an increase is not observable. The persistence of single rounded intracorpuscular parasites (O), following upon the administration of the drug, may be due to some extent to the after-effects of the drug upon free pyriform parasites whose sojourn in the plasma may have been so brief as not to affect their vitality to too great a degree. On the other hand, it is certain that, in the normal course of events, a number of round parasites degenerate and die before giving rise to pyriform parasites. Again, there can be no doubt that in some cases their development into pyriform parasites may be delayed by unfavourable conditions. The delay cannot, however, be indefinite since, to survive, the parasite must attain the pyriform stage before the degeneration of the containing corpuscle takes place. We know from the observations of Nuttall and Graham-Smith upon living parasites that the stages of the parasite other than the pyriform have always been seen to promptly degenerate and die upon their escape into the plasma.

It is worthy of note in this connection that *P. canis* assumes a rounded form when it dies. This can be observed in fresh preparations under the microscope, in blood kept *in vitro* and in blood taken from dogs some hours after death.

The fall in the total number of infected corpuscles which accompanies or ultimately follows the disappearance of the (PP) parasites also indicates that the action of the drugs is directed primarily against the pyriform parasites. It is the latter stage of the parasite which invades fresh corpuscles.

In this connection the protocols relating to Dogs 12 and 10 are of interest. In the first instance the drug caused a considerable reduction of (PP) parasites, although it was given on the day when the dog died. Dog 12 had not been previously treated. On the other hand, when the relapse occurred in Dog 10, which had been previously treated, a second dose of Trypanblau seemed to exert no influence on the parasites. Although this observation requires confirmation by further experiment, it certainly suggests that the parasites which survive the first dose of the drug may have become resistant to its action. That trypanosomes may acquire a considerable resistance to the effects of drugs like those we have used has been clearly established.

We shall, however, leave the further consideration of this subject for a future paper, in which we shall deal with observations upon the parasites under the microscope, and with the effects exerted by the drugs upon their morphology.

The enumeration of parasites in treated dogs.

We have already stated that the chief effect of Trypanblau and Trypanrot is to cause the disappearance of the pyriforms and a relative increase in the percentage of rounded parasites. Accompanying the fall in the relative number of pyriform parasites there is also a fall in the percentage of infected as compared to uninfected corpuscles. That this is the case is best shown by the following enumerations taken from our protocols.

For instance, in the case of Dog 2, prior to its being treated on the 11th day after inoculation, for every 100 (O) parasites there were present the following numbers of (PP) parasites:

	Variation within 14 hours on day 7				Variation within 13 hours on day 8				Variation on days 9		10	11
% of (PP)	122	83	140	74	82	58	104	78	42	54	50	44
% of infected r.b.c.	?	?	?	0·1	?	0·4	0·4	1·5	5	5·6	6·4	6

The sign "?" indicates that there were too few parasites present to determine the percentage.

This dog was treated with a small dose of *Trypanrot* on the 11th day, with the result that the number of (PP) parasites and the percentage of infection both fell as follows, all parasites having disappeared on the 15th day:

Day	11	12	13	14	15
Intervals of	$22\frac{1}{2}$ hours	24 hours	24 hours	24 hours	
% of (PP)	44 T	30	13	9	0
% of infected r.b.c.	6	2·6	2·2	?	0

("T" indicates when the drug was given.)

The following extracts from the protocols of four other dogs show a similar effect of a dose of *Trypanblau*, the order being according to the degree of infection of the corpuscles at the time when the drug was given:

Record of Dog 10.

Intervals of	$3\frac{1}{2}$ hrs.	$2\frac{1}{2}$ hrs.	2 hrs.	$2\frac{1}{2}$ hrs.	$2\frac{1}{2}$ hrs.	$2\frac{1}{2}$ hrs.	1 hr.	$6\frac{3}{4}$ hrs.	$2\frac{3}{4}$ hrs.	
% of (PP)	68	78 T	32	13	0·5	2	0·5	0	0·5	0
% of infected r.b.c.	1·5	4·5	3·8	7*	3·1	2·7	2	1·5	0·1	?

* This high % was doubtless due to massage of the ear to promote blood flow after puncturing the carvein.

Record of Dog 11.

Intervals of	$4\frac{1}{2}$ hours	$2\frac{1}{2}$ hours	2 hours	$2\frac{1}{2}$ hours	$2\frac{1}{2}$ hours
% of (PP)	105	125 T	55	16	3·7	0
% of infected r.b.c.	1·1	3·8	3·7	3·6	1·4	4*

Note:—The % of infected r.b.c. fell slowly in this case and at a later period reached 0. The high % marked by * was due to massage of the ear.

Record of Dog 4.

Intervals of	2 hours	3 hours	3 hours	3 hours	3 hours
% of (PP)	100	69 T	1	1	1	0
% of infected r.b.c.	3·5	3·4	1·2	0·8	0·4	?

Record of Dog 3.

Intervals of	2 hours	3 hours	$\frac{1}{2}$ hour	$2\frac{1}{2}$ hours
% of (PP)	92	62 T	0	3	0
% of infected r.b.c.	0·2	?	?	?	?

Even in the case of Dog 12, where Trypanblau was given only 3 hours before death took place from piroplasmosis, the drug exerted an effect upon the parasites, for of 100 parasites counted immediately before the injection of the drug 57·5 % were (PP) and 32·5 % were (O), whereas $2\frac{3}{4}$ hours after the injection there were only $22\frac{1}{2}$ % (PP) as against $77\frac{1}{2}$ % (O) found in the blood films.

In the following tables we give a summary of the effect of Trypanblau (TB.) and Trypanrot (TR.) upon *Piroplasma canis*, citing only the experiments where accurate counts were made of the different types of parasites.

In Table I, the number of the dog, its description and weight are recorded in the first column. In the second column is given the dose of the drug which was injected into the dog, the dose being reckoned per ounce (= 28·35 grammes) of dog in terms of saturated watery solution. In the third column is given the percentage of infected corpuscles, the counts having been made immediately before the injection of the drug. In the fourth column is given the reduced percentage of infected corpuscles consequent upon the injection of the drug, the times when these counts were made being reckoned from the moment when the drugs were injected. Where the sign "?" occurs in the fourth column, it means that the number of parasites was so small as to exclude the possibility of determining the percentage of infected corpuscles, for the

reason that only a few infected corpuscles could be detected at the edge of the film. In the fifth column the time is stated when the parasites could no longer be detected in blood films by microscopic observation.

TABLE I.

Dog	No. and description	Dose of drug given per oz. of dog, c.c. of sat. solution	% of infected r.b.c. At time when drug was given	% of infected r.b.c. At stated intervals of time after drug was given	Time it took for all parasites to disappear after drug injection	Remarks
2.	Collie: ¾ grown, wt. 20 lbs.	·012 TR.	6	? after 70 hrs.	96 hrs.	Advanced case, died.
3.	Terrier: adult, wt. 17½ lbs.	·016 TB.	0·2	? after 6 hrs.	22 hrs.	Recovered.
4.	Terrier: adult, wt. 20 lbs.	·017 TB.	3·4	1·2 after 3 hrs.	47 hrs.	Recovered.
9.	Terrier: pup, wt. 7 lbs.	·032 TB.	v. few	? after 6 hrs.	42 hrs.	Recovered.
10.	Terrier: pup, wt. 1 lb. 11 oz.	·037 TB.	4·5	2·7 after 9 hrs.	20 hrs.	Both died from relapse. See note to protocols on p. 186.
11.	Terrier: pup, wt. 1 lb. 4 oz.	·037 TB.	3·8	1·4 after 7 hrs.	20 hrs.	

Dog 2 was treated in an advanced stage of the disease on the 6th day after the parasites had appeared, and when 6 % of the red blood corpuscles were infected. This dog ultimately died, but no parasites could be found in its body at autopsy. Dogs 3, 4 and 9 received treatment 24—25 hours after the parasites first appeared in their blood; all three recovered. Dogs 10 and 11 were inoculated on the same day when the parasites first appeared in their blood; they died of piroplasmosis.

If we summarize the effect of the drug in causing the disappearance of the (PP) parasites alone we find the following results were obtained, the percentage of (PP) parasites being reckoned in terms of the percentage of (O) parasites, as stated on page 161.

TABLE II.

Dog No.	The % of (PP) parasites in blood-films: At time when drug was given	Reduction at stated intervals of time after drug was given	Time when all (PP) parasites disappeared
2*	40	9 after 70 hrs.	96 hrs., advanced case.
3	62	0 after 6 hrs.	6 hrs., recovered.
4	69	1 after 3 hrs.	11 hrs., recovered.
9	125	2 after 6 hrs.	20 hrs., recovered.
10	78	2 after 9 hrs.	21 hrs., died.
11	125	3·7 after 7 hrs.	9 hrs., died.

* TR. treated.

In Table III, we record the results obtained on all the dogs that were treated, including (*a*) the day after inoculation when the parasites first appeared in the blood, (*b*) the day the dog received treatment reckoned from the time when the parasites first appeared, (*c*) the time when the parasites disappeared after the first injection of the drug, and (*d*) the time when the parasites reappeared after their first disappearance, following the injection of the drug:

TABLE III.

No. of Dog	*a* Day after inoculation when parasites first appeared	*b* Day when dog was treated after parasites appeared	*c* Time it took the parasites to disappear after drug was injected	*d* Time when parasites reappeared in days (after disappearance)	Result
2*	11	6	96 hrs.	—	Died, but no parasites found at autopsy.
3	7	1	22 ,,	11	Lived.
4	7	1	47 ,,	10	Lived.
9	6	1	– 42 ,,	12	Lived 51 days; death attributed to severe mange; see protocol, p. 182.
10	4	same day	20 ,,	4	Died } See note following
11	4	,,	20 ,,	5	Died } protocol on p. 186.
8⊙	5	,,	24 ,,	9	Lived 43 days. Died of distemper.
5	4	1	– 48 ,,	9	Lived.
6	6	same day	– 48 ,,	10	Lived.
7†	5	,,	– 48 ,,	19	Lived.

* Trypanrot treatment. ⊙ Received 3 doses of Trypanblau.

† Received 2 doses with 1 day's interval between the injections.

– before the numbers in column (*c*) indicates that the parasites may have disappeared earlier, *i.e.* during the preceding 24 hours.

In the above table we have not included Dog 1, which was treated with Trypanrot and in which the parasites disappeared and reappeared irregularly. This dog lived.

It will be noted that in the two Dogs 10 and 11, in which a relapse occurred, the parasites reappeared after a shorter interval than in any of the other animals.

Records of Experiments.

The Controls.

The experiments recorded in the following protocols were conducted upon 21 dogs, of which 8 served as controls. That we were dealing with

a very fatal disease is sufficiently evidenced by the protocols relating to the control animals, all of which developed fatal piroplasmosis: 5 died a natural death from the disease and 3 were killed whilst moribund.

The protocol relating to Control Dog VIII includes a detailed account of the blood examination up to the time of death:

Control Dog I. Large Irish terrier, mongrel. Weight 18 lbs.

	Day	
(1. III. 09.)	1.	*Inoculated* with 5 c.c. of heart blood.
	5.	Parasites found in blood films.
	6.	Parasites occupy nearly all the corpuscles.
	7.	Dog very ill. Haemoglobinuria.
(8. III. 09.)	8.	*Dog died*, with many parasites in its blood.

Control Dog II. Irish terrier. Weight about 15 lbs.

	Day	
(1. III. 09.)	1.	*Inoculated* with 5 c.c. of heart blood.
	5.	Parasites found in blood.
	6.	Fifteen parasites per field.
	7.	Many parasites.
	8.	Dog ill.
(9. III. 09.)	9.	*Dog died*, with many parasites in its blood.

Control Dog III. Mongrel Sheep dog. Weight 16 lbs.

	Day	
(8. III. 09.)	1.	*Inoculated* with 4 c.c. of heart blood.
	8.	Parasites appear, a few (PP).
	9.	Parasites increasing in numbers.
	10.	Dog not well. Parasites increased.
	11.	Dog ill. Parasites more numerous.
	12.	Dog very ill. Haemoglobinuria.
(20. III. 09.)	13.	*Dog killed when moribund.* Parasites 10 per field.

Control Dog IV. Irish terrier. Weight about 19 lbs.

	Day	
(20. III. 09.)	1.	*Inoculated* with 7 c.c. of heart blood.
	3.	Parasites appear in blood, 2 (PP) found in blood-film.
	4.	A few pyriform parasites found.
	5.	Dog slightly ill; 2—3 infected r.b.c. per field.
	6.	Dog ill; 10—12 infected r.b.c. per field. Haemoglobinuria.
(26. III. 09.)	7.	*Dog dying of piroplasmosis.* Killed.

Control Dog V. Adult mongrel Sky terrier. Weight about 14 lbs.

	Day	
(7. IV. 09.)	1.	*Inoculated* with 6 c.c. of heart blood.
	8.	Dog ill. Haemoglobinuria.
(15. IV. 09.)	9.	*Dog died* of piroplasmosis.

Control Dogs VI and VII.

These dogs served as controls to Dogs 6 and 7 which were treated with Trypanblau and recovered. Both of the control animals had been treated with blood containing dead *P. canis* in the hope of producing immunity in them. They were both inoculated with virulent blood nine days after the last injection with blood containing dead *P. canis*.

		Day	
(15. iv. 09.)	Dog VI.	1.	*Inoculated* with 5 c.c. of virulent blood.
		6.	Parasites appeared in its blood.
		8.	Dog died of piroplasmosis. Haemoglobinuria. Many parasites were present, 29 % of the r.b.c. being infected.
		Day	
(15. iv. 09.)	Dog VII.	1.	*Inoculated* with 5 c.c. of virulent blood.
		6.	Parasites appeared in its blood.
		8.	5 % of r.b.c. infected.
		9.	14 % ,, ,,
		10.	5 % ,, ,, ; haemoglobinuria, very weak.
		11.	2 % ,, ,,
		12.	Few parasites.
		13.	0·8 % of r.b.c. infected; very anaemic.
		29.	Had gradually picked up during the intervening days there being no parasites on some days, few on others. At one time they were absent for a period of 9 days (days 21–28). The dog seemed to be recovering when the parasites reappeared on the 29th day.
		30.	1 % of r.b.c. infected.
		31–32.	Few infected r.b.c. at film-edge.
		33.	2 % of r.b.c. infected.
		34.	1 % ,, ,,
		35.	0·5 % ,, ,,
(20. v. 09.)		36.	— *Dog died* of chronic piroplasmosis.

Note:—The full account of Dog VII will be found in the protocols accompanying the paper by Nuttall and Graham-Smith which will appear in the next number of *Parasitology*.

Control Dog VIII. Large mongrel collie. Weight 39¼ lbs.

	Day		Temp. ° F.	% of infected r.b.c. (1000 counted)	The infected r.b.c. contained parasites as follows: % P	% D	% O	% PP	% OO	% PPPP
(29. iii. 09.)	1.	*Inoculated* with 5 c.c. of heart blood.								
	4.	*Parasites appear* in blood, very few at film-edge	—	+	0	0	0	+	0	0
	5.	,, ,, ,, ,,	104·4	+	0	2	60	35	1	2
	6.	Parasites increasing 1 a.m.	—	0·6	1	4	64	30	0	1
		3 a.m.	104·5	0·4	0	3	54	43	0	0
		6 a.m.	104·5	1·0	0	1	61	37	0	1
		9.15 a.m.	105·2							
		9.45 a.m.	—	2·0	0	2	56	42	0	0
		1.15 p.m.	105·4	1·2	0	1·5	49	46	1	1·5
		4 p.m.	—	2·8	3	4	41	50	1	1
(4. iv. 09.)	7.	Parasites increasing.								
		Haemoglobinuria 11.30 a.m.	105·1	17	1	2·5	63·5	30	2	1
		Dog moribund, killed 6 p.m.	104·7	28	0	5	53	41	·5	·5

Note:—The sign + indicates that there were too few infected corpuscles present to permit of a determination of their percentage.

Records of treated dogs.

Experiments with Trypanrot.

We only carried out two experiments with Trypanrot. In the first experiment (*Dog* 1) the drug was given on four occasions. This dog developed a very mild and chronic form of the disease, only appearing ill at the commencement. The dog appeared well and lively after the 16th day from the time of inoculation. No parasites were to be found in blood-films between the 15th and 28th days, after which they appeared irregularly in small numbers at the edge of the film only, as will be seen by reference to the protocol. The animal is still alive (111 days on 17. VI. 09), whereas the *Control Dog* II died on the 9th day after inoculation. This being one of our earlier experiments, the protocol does not include a detailed enumeration of infected corpuscles.

In the second experiment (*Dog* 2) the drug was given 6 days after the parasites appeared in the blood at a time when 6 % of the corpuscles were infected. After 24 hours there were 2·6 % of infected corpuscles, and the dog had haemoglobinuria. *No parasites could be subsequently detected,* but nevertheless the dog died on the 20th day, apparently from the after-effects of the disease. The *Control Dog* VIII died on the 7th day after inoculation.

Dog 1.

Terrier, about 2 years old and of slight build. Weight 15 lbs. Received four doses of drug.

	Day	
(1. III. 09.)	1.	*Inoculated* with 5 c.c. of heart blood.
	5.	A few *parasites appeared* in the blood.
		Treatment begun: 6 c.c. of 1 % Trypanrot injected subcutaneously.
	6.	15 infected r.b.c. per field, many (PP).
	7.	Dog lively; 5 infected r.b.c. per field; no (PP), only (O).
		Treatment: 6 c.c. of 1 % Trypanrot as before.
	8.	Dog not well; 10 infected r.b.c. per field, some (PP).
	9.	Dog ill; 10 infected r.b.c. per field, some (PP).
		Treatment: 6 c.c. of 1 % Trypanrot as before. Blood examined 4 hours after administration of drug; only (O) parasites found in film.
	10.	Dog fairly well, 1—2 infected r.b.c. per field; a few (PP).
	11.	Dog better. Parasites hard to find.
	12.	Dog much better; eats well. Infected r.b.c. very scarce, 5 (PP) found after much searching.

Day		
13.		Dog improving; 1 infected r.b.c. found in film.
15.		Dog nearly well; weight 14 lbs. No parasites found.
16.		Dog well. No parasites found.
		Treatment: 5 c.c. of 1 % Trypanrot as before.
17—28.		Dog continues well. Daily examination of blood-films revealed no parasites.
29.		Dog well; 4 infected r.b.c. [2 (O), 1 (PP)] found in blood-film after much searching.
33.		Dog lively and well. Weight 14 lbs.
34.		Temp. normal. 6 infected r.b.c. found in blood-film.
36.		,, ,, Ditto.
37.		,, ,, No parasites.
39—40.		,, ,, No parasites.
42.		,, ,, 4 infected r.b.c. found: 3 (PP), 1 (PPPP).
43.		,, ,, 2 ,, ,, 1 (O), 1 (PPPP).
44—47.		,, ,, No parasites.
48.		,, ,, 1 infected r.b.c. found: 1 (O).
49.		,, ,, 1 ,, ,, 1 (OOOO).
50—51.		,, ,, No parasites.
	Temp. °F.	
52.	102·5.	4 infected r.b.c.: 1 (PP), 2 (PPPP), 1 (O). Weight 14½ lbs.
53.	102.	3 ,, ,, 2 (PP), 1 (PPPP).
55.	101.	1 ,, ,, 1 (P).
57.	101·3.	3 ,, ,, 1 (PPPP), 1 (O), 1 (DD).
60.	103.	2 ,, ,, 1 (PP), 1 (OOOO).
63.	101·4.	No parasites. Dog lively.
67.	102.	3 infected r.b.c.: 2 (O), 1 (OO).
70.	101·1.	5 ,, ,, 2 (O), 3 (PP).
74.	100·4.	1 ,, ,, 1 (PPPP).
76.	103·4.	No parasites.
77.	—	No parasites.
81.	104·4.	9 infected r.b.c.: 2 (PP), 2 (PPPP), 5 (O). Weight 13¾ lbs.
84.	104·4.	10 ,, ,, 5 (PP), 5 (O).
85.	103·4.	2 ,, ,, 2 (PP).

After this date the dog's temperature remained normal and no more parasites could be found. Weight on 101st day = 15½ lbs. The dog continues to look healthy to date (17. VI. 09), the 111th day.

Note:—Dog not examined on days 14, 38, 41, 54, 56, 58, 59, 61, 62, 64, 65, 66, 68, 69, 71, 72, 73, 75 etc. (numbers omitted from protocol).

Control Dog II (see p. 170) died on the 9th day after inoculation.

Dog 2.

Mongrel collie, ¾ grown, under 1 year old. Weight 20 lbs. One dose of drug given when the disease was well advanced and there appeared to be no chance of recovery.

	Day		Temp. °F.	% of infected r.b.c. (1000 counted)	The infected r.b.c. contained parasites as follows					
					% P	% D	% O	% PP	% OO	% PPPP
(27. III. 09.)	1.	*Inoculated* with 6 c.c. of heart blood.								
	6.	*Parasites appeared* in blood at 10 a.m.	103·6	+						
		Very few at edge of film at 3.15 a.m.	103·2	+	0	0	+	+	0	0
		6 p.m.	103·6							
	7.	Few parasites at edge of film at 10 a.m.	104·2	+	0	0	+	+	0	0
		Ditto at 12 noon	104·6	+	0	0	+	+	0	0
		Few parasites all over film at 3 p.m.	105·0	+	0	0	36	44	0	20
		Ditto at 6 p.m.	105·4	+	4	0	48	40	4	4
		Ditto at 9 p.m.	104·3	+	0	0	40	56	0	4
		Ditto at 12 midnight ...	104·3	0·1	0	2	54	40	2	2
	8.	3 a.m.	104·5	+	0	2	51	42	1	4
		6 a.m.	104·5							
		9.45 a.m.	102·3	0·4	0	0	62	36	1	1
		1 p.m.	105·4	0·4	0	2	46	48	1	3
		4 p.m.	105·4	1·5	0	5	52	41	2	0
	9.	Many parasites, 11.30 a.m. ...	105·9	5	0	1	68·5	29·5	1	0
		6 p.m.	104·7	5·6	0	4	61	33	2	0
	10.	10 a.m.	105·8	6·4	0	1	66	33	0	0
	11.	*Treatment:* 7½ c.c. of 1 % Trypanrot, 11.30 a.m.	105·9	6	0	2	68	30	0	0
	12.	Haemoglobinuria and great weakness, 10 a.m.	105·2	2·6	0	0	76	23	1	0
	13.	Slightly better, 10 a.m. ...	103·8	2·2	0	0	87	11	2	0
	14.	Do., fewer parasites, a.m. ...	101·4	+	0	0	88	8	3	1
	15.	Improving, no parasites, a.m.	100·4	0	0	0	0	0	0	0
	16.	No parasites, a.m.	100·7	0	0	0	0	0	0	0
	17.	,, ,, a.m.	101·6	0	0	0	0	0	0	0
	18.	,, ,, a.m.	101·4	0	0	0	0	0	0	0
	19.	,, ,, dog very ill, a.m.	101·6	0	0	0	0	0	0	0
(15. IV. 09.)	20.	Dog *died.*								

Note:—The signs + in the columns to the right indicate that the percentage of infected corpuscles was too small to determine without counting many thousand corpuscles.

Control Dog VIII (see p. 171) died on the 7th day after inoculation.

Experiments with Trypanblau.

Owing to the fact that Trypanblau appeared to exert a more marked effect upon the parasites than did Trypanrot, we conducted the rest of our experiments with Trypanblau. Of the 10 dogs which underwent *curative* treatment, seven made a good recovery. Three of these dogs are still alive (17. vi. 09), having been respectively under observation for periods of 90, 83 and 65 days. Four of the recovered dogs subsequently died of intercurrent disease (distemper mostly, and mange) having lived respectively for 42, 43, 52 and 69 days after they had been inoculated. Three of the treated dogs died of piroplasmosis, namely, a dog (12) treated on the day of its death and which was to be regarded as a hopeless case at the start, and dogs 10 and 11, which died of a relapse. The last-named dogs were very poor subjects for experiment, as will be seen by reference to the protocols.

Finally, one dog (13) received a dose of the drug before the parasites would naturally have appeared, that is, 24 hours after inoculation with virulent blood. This dog never showed parasites in its blood, and it remained perfectly healthy up to the 65th day after inoculation.

On the other hand, all of the dogs (7) which served as controls for these experiments, died of piroplasmosis.

The dogs which received curative treatment were as follows:

Dog No.	% of infected r.b.c. at time of treatment	No. of doses of drug given	Result of treatment	No. Control dogs
3	0·2	1	Living 90th day	"IV" died 7th day.
4	3·5	1	,, 83rd ,,	"VIII" died 7th day.
5	0·4	1	Lived 69 days, when it died of distemper and mange	"V" died 9th day.
6	0·6	1	Living 65th day	"VI" died 8th day.
7	0·025	2	Lived 42 days, when it died of distemper	"VII" died 36th day.
8	?	4	Lived 43 days, when it died of distemper	"I" died 8th day.
9	?	2	Lived 52 days, when it died from mange	"IV" died 7th day.
10	4·5	2	Died 13th day piroplasmosis relapse	"VIII" died 7th day.
11	3·8	2	Died 15th day piroplasmosis relapse	,, ,, ,,
12	50	1	Died 3 hours after treatment, but parasites visibly affected by drug.	

Note:—Whereas all the (7) controls died of piroplasmosis only 3 of the 10 treated dogs (Nos. 10, 11, 12) died from this cause.

Dog 3.

Adult Irish terrier. Weight 17½ lbs. Received one dose of drug.

	Day		The infected r.b.c. contained parasites as follows					
			% P	% D	% O	% PP	% OO	% PPPP
(20. III. 09.)	1.	*Inoculated* with 5 c.c. of heart blood.						
	6.	2 (O) *parasites found* after much searching.						
	7.	At 10 a.m. few parasites in blood	0	0	52	48	0	0
		12 noon ·02 % of r.b.c. infected	2	2	58	36	0	1
		Treatment: 4½ c.c. of sat. sol. Trypanblau.						
		3 p.m. few parasites ...	0	0	100	0	0	0
		3.30 p.m. ,, ,, ...	0	0	96	3	1	0
		6 p.m. very few parasites (only 40 found in film)	0	0	100	0	0	0
	8—15.	No parasites. Dog lively and well	0	0	0	0	0	0

		Temp. °F.	
	16.	103·1.	No parasites.
	17.	102·6.	,, ,,
	18.	102·6.	,, ,,
	19.	102·7.	3 infected r.b.c.: 2 (PP), 1 (O).
	20.	102·2.	No parasites.
	21.	102·4.	1 infected r.b.c.: (PP).
	22.	103·6.	4 ,, ,, 4 (O).
	23.	103·7.	7 ,, ,, 6 (O), 1 (PP).
	24.	102·8.	No parasites.
	25.	102·5.	,, ,,
	26.	102·2.	,, ,,
	27.	102·0.	,, ,,
	28.	102·0.	,, ,,
	29.	101·5.	,, ,,
	30.	102·4.	,, ,,
	31.	101·7.	,, ,,
	32.	103·1.	,, ,, Weight 17¾ lbs.
	33.	102·2.	,, ,,
	35.	101·0.	,, ,,
	37.	102·0.	,, ,,
	40.	103·9.	,, ,, Rise due to excitement ? (fighting).
	43.	103·6.	,, ,, Dog lively.
	46.	101·4.	,, ,, Dog very well.
	50.	104·0.	A few parasites at film-edge.
	51.	102·6.	7 infected r.b.c.: 2 (PP), 5 (O).
	52.	103·2.	2 ,, ,, 2 (PP).
	53.	102·0.	7 ,, ,, 6 (O), 1 (PP).
	54.	102·6.	1 ,, ,, 1 (PP).
(13. v. 09.)	55.	102·4.	No parasites.

After this date the dog's temperature remained normal but for a rise on the 77th day (104° F.). No parasites could be found in blood films after the 55th day. On the 80th day the dog weighed 17¼ lbs. The dog continues lively and well to date (17. VI. 09), the 90th day.

Note:—Dog not examined on days 34, 36, 38, 39, 41, 42, 44, 45, 47, 48, 49.

Control Dog IV (see p. 170) died on the 7th day after inoculation.

Dog 4.

Large Fox terrier. Weight 20 lbs. Received one dose of drug on 7th day.

	Day	Temp. °F.		% of infected r.b.c. (1000 counted)	Infected r.b.c. contained parasites as follows					
					% P	% D	% O	% PP	% OO	% PPPP
(27. III. 09.)	1.		*Inoculated* with 6 c.c. of heart blood.							
	6.	—	*Parasites appeared* in blood.							
		102·8.	Very few at film-edge at 10 a.m.							
		102·0.	... ... 3.15 p.m.	+						
		102·6.	... ... 6 p.m.							
	7.	104·2.	... ... 10 a.m.	3·5	0	0	50	50	0	0
			Treatment: 5·5 c.c. of sat. sol. Trypanblau							
		104·2.	Trypanblau at 12 noon	3·4	0	0	58·8	40	·05	1
		105·2.	... ... 3 p.m.	1·2	0	0	98	1	1	0
		105·4.	... ... 6 p.m.	0·8	0	0	99	1	0	0
		105·1.	... ... 9 p.m.	0·4	0	0	99	0	1	0
		104·9.	Few parasites at film-edge at 12 midnight.	+	0	0	100	0	0	0
	8.	105·9.	Very few parasites all over film at 3 a.m.	+	0	0	100	0	0	0
		104·6.	Few parasites at film-edge 6 a.m.	+	0	0	100	0	0	0
		104·6.	Very few ,, ,, 9·45 a.m.	+	0	0	100	0	0	0
		104·2.	,, ,, ,, 1 p.m.	+	0	0	100	0	0	0
		104·5.	,, ,, ,, 5 p.m.	+	0	0	100	0	0	0
	9.	102·0.	No parasites found 11.30 a.m.	0						
	10.	102·9.	,, ,, ,, 10 a.m.	0						
	11.	101·5.	,, ,, ,, 11.30 a.m.	0						
	12.	101·6.	,, ,, ,, ,,	0						
	13.	101·8.	,, ,, ,, ,,	0						
	14.	102·2.	,, ,, ,, ,,	0						
	15.	102·8.	,, ,, ,, ,,	0						
	16.	102·0.	,, ,, ,, ,,	0						
	17.	102·4.	,, ,, ,, ,,	0						
	18.	102·4.	,, ,, ,, ,,	0						
	19.	102·4.	8 infected r.b.c. found, 7 (O), 1 (DD).	+						
	20.	104·1.	9 infected r.b.c. found, 6 (PP), 3 (O).	+						
	21.	103·2.	6 infected r.b.c. found, 5 (O), 1 (PP).	+						
	22.	101·3.	No parasites found	0						
	23.	103·8.	,, ,, ,,	0						
	24.	102·7.	2 infected r.b.c.: 2 (O)	+						
	25.	103·9.	No parasites found. Wt. 21¼ lbs.	0						
	26.	102·2.	,, ,, ,,	0						
	28.	100·8.	1 infected r.b.c.: 1 (PP).							
	30.	103·1.	Several infected r.b.c.: (PP) and (O).							

Day	Temp. °F.	
31.	103·0.	4 infected r.b.c. : 2 (PP), 2 (O).
33.	103·8.	8 ,, ,, 6 (PP), 2 (O).
36.	102·2.	8 ,, ,, 7 (O), 1 (PPPP). Dog gaining in weight.
39.	102·4.	No parasites.
42.	102·8.	7 infected r.b.c. : 4 (PP), 3 (O). About 30 c.c. of blood taken from earvein.
43.	101·8.	No parasites.
44.	100·4.	1 infected r.b.c. : 1 (O).
45.	102·6.	5 ,, ,, 1 (PPPP), 4 (O).
46.	101·4.	A few infected r.b.c. at film-edge (O), (PP), about 1 per 3 fields.
47.	100·6.	6 infected r.b.c. : 2 (PP), 4 (O).
48.	101·4.	9 ,, ,, 3 (PP), 6 (O).
(14. v. 09.) 49.	101·6.	No parasites.

After this date the dog's temperature remained normal but for slight rises (103·4° and 103·2° F.) on the 69th and 72nd days respectively. No parasites were detected in the blood after the 49th day. On the 73rd day the dog weighed 17¾ lbs. The dog continues lively and well to date (17. vi. 09), the 83rd day.

Note:—Dog not examined on days 27, 29, 32, 34, 35, 37, 38, 40, 41. This dog never appeared to be ill.

Control Dog VIII (see p. 171) died on the 7th day after inoculation.

Dog 5.

Adult Fox terrier. Weight 13¼ lbs. Received one dose of the drug.

	Day	Temp °F.	
(7. iv. 09.)	1.	—	*Inoculated* with 6 c.c. of heart blood.
	4.	—	*Parasites appeared:* 3 infected r.b.c.: 2 (PP), 1 (O) at film-edge.
	5.	101·0.	0·4 % of r.b.c. infected (500 counted). *Treatment:* 5 c.c. of sat. sol. Trypanblau at mid-day. Dog became very blue.
	6.	104·7.	2 infected r.b.c. found at film-edge (10 a.m.). Swelling at seat of drug injection.
	7.	101·2.	No parasites. Dog lively; swelling lanced, sero-pus escaping.
	8.	101·5.	,, ,, ,, ,, diminishing.
	9.	101·0.	,, ,, ,, ,, practically gone.
	10.	102·2.	,, ,,
	11.	101·6.	,, ,,
	12.	102·0.	,, ,,
	13.	101·7.	,, ,,
	14.	103·0.	,, ,, Dog weighed 12¾ lbs.
	15.	102·7.	,, ,,
	16.	101·1.	3 infected r.b.c. found at film-edge: 2 (PP), 1 (O).
	17.	101·8.	No parasites.
	18.	101·8.	,, ,,
	19.	101·8.	,, ,,
	20.	101·5.	,, ,,
	21.	101·7.	,, ,,
	22.	102·7.	5 infected r.b.c. at film-edge: 3 (PP), 2 (O).
	23.	101·9.	8 ,, ,, ,, 7 (PP), 1 (O).
	24.	101·0.	3 ,, ,, ,, 3 (PP).
	25.	100·9.	6 ,, ,, ,, 5 (PP), 1 (O).
	26.	101·3.	5 ,, ,, ,, 4 (PP), 1 (O).
	27.	103·0.	No parasites.
	28.	102·0.	1 infected r.b.c. at film-edge: 1 (PP).
	29.	Normal.	No parasites.
	30.	101·2.	,, ,,
	31.	100·8.	,, ,,
	32.	101·0.	,, ,,
	36.	101·0.	,, ,,
(13. v. 09.)	38.	102·4.	,, ,,

After this date rises of temperature were recorded on the 42nd (103·2° F.), 45th (106°), 46th (104°), and 58th (103·4°) days. Parasites were only discovered once at the edge of a blood film, 2 (O), on the 56th day, after which they could no longer be detected. On the 42ud day the dog weighed 13¼ lbs. On the 57th day severe *mange* set in and the animal rapidly became ill and emaciated. On the 67th day it developed *distemper* with copious discharges from the nose and eyes. The dog died of distemper and mange on the 69th day, its weight having fallen to 7¼ lbs. *No Piroplasma* could be found at autopsy (14. vi. 09).

Note :—Dog not examined on days 33, 34, 35, 37.

Control Dog V (see p. 170) died on the 9th day after inoculation.

Dog 6.

Young mongrel terrier. Weight 16 lbs. Received one dose of the drug.

	Day	Temp. °F.	
(15. IV. 09.)	1.	—	*Inoculated* with 5 c.c. of heart blood.
	6.	103·1.	*Parasites appeared:* 0·6 % of r.b.c. infected. *Treatment:* 6 c.c. of sat. sol. Trypanblau.
	7.	104·7.	2 (O) infected r.b.c. at film-edge. Painful swelling at seat of drug injection.
	8.	103·2.	No parasites.
	9.	105·0.	,, ,,
	10.	103·8.	,, ,,
	11.	104·0.	,, ,, Slough at seat of drug injection.
	12.	101·6.	,, ,, Dog lively; seat of drug injection better.
	13.	101·4.	,, ,, ,, ,,
	14.	101·8.	,, ,, ,, ,,
	15.	101·1.	,, ,, ,, ,,
	16.	101·6.	,, ,, ,, ,,
	17.	100·7.	,, ,, ,, ,,
	18.	102·0.	1 infected r.b.c.: 1 (PP).
	19.	102·0.	1 ,, ,, 1 (O).
	20.	102·2.	No parasites.
	21.	101·4.	,, ,,
	22.	101·3.	,, ,,
	23.	101·2.	,, ,,
	24.	101·8.	,, ,,
	28.	101·6.	,, ,,
	30.	103·3.	,, ,,
	35.	103·8.	8 infected r.b.c.: 2 (PP), 5 (O), 1 (OOOO). Weight 14¾ lbs. Coughing.
	38.	104·6.	1 infected r.b.c. per field.
	39.	104·4.	No parasites.
	42—43.	103·0.	,, ,,
	44—48.	Normal.	No parasites.
	49.	,,	Very few parasites at film-edge.
(16.VI.09.)	50—64.	,,	No parasites.

This dog only showed parasites on one day after the 39th day, and its temperature remained normal until the 64th day. On the 55th day it showed symptoms of *distemper* from which it apparently recovered. The dog looks lively and well at present, 65th day (17. VI. 09).

Note:—Dog not examined on days 25, 26, 27, 29, etc.

Control Dogs VI and VII (see p. 171). No. VI died on the 8th day after inoculation, No. VII died of piroplasmosis on the 36th day after inoculation.

Dog 7.

Young Dane. Weight 57¼ lbs. Received two doses of drug.

	Day	Temp. °F.	
(15. iv. 09.)	1.	...	*Inoculated* with 5 c.c. of heart blood.
	4.	103·0.	No parasites.
	5.	103·0.	*Parasites appeared*, only ·025 % r.b.c. infected. *Treatment:* 10 c.c. of sat. sol. Trypanblau at 6 p.m.
	6.	105·1.	A few (O) infected r.b.c. at film-edge at 5 p.m.
	7.	103·8.	No parasites. *Treatment:* 10 c.c. of sat. sol. Trypanblau.
	8.	105·2.	,, ,,
	9.	105·1.	,, ,,
	10.	103·2.	,, ,,
	11.	103·0.	,, ,, Slough at seat of drug injection.
	12.	102·3.	,, ,,
	13.	102·9.	,, ,,
	14.	104·5.	,, ,, Fever due to *distemper*.
	15.	105·6.	,, ,, Distemper: discharging from nostrils.
	16.	104·4.	,, ,, Dog seems better.
	17.	104·6.	,, ,,
	18.	103·6.	,, ,,
	19.	102·2.	,, ,, Dog improving.
	20.	101·0.	,, ,, Much better.
	21.	101·2.	,, ,,
	22.	101·0.	,, ,,
	23.	101·0.	,, ,,
	24.	101·2.	2 infected r.b.c.: 2 (O).
	25.	99·9.	No parasites.
	26.	101·0.	4 infected r.b.c.: 3 (O), 1 (PP).
	27.	101·2.	No parasites.
	29.	104·8.	,, ,,
	30.	103·6.	,, ,,
	31.	102·4.	,, ,,
	32.	103·8.	,, ,, Dog coughing.
	33.	103·4.	,, ,,
	34.	104·0.	6 infected r.b.c., all (O). Weight 51 lbs., 9 oz.
	35—36.	104·2.	No parasites, still coughing.
	37.	105·6.	,, ,, Dog ill.
	38.	106·5.	,, ,, *Distemper:* running at eyes and nose. Very ill.
	41.	103·4.	,, ,, ,, ,, ,, ,, ,,
(26. v. 09.)	42.	—	Dog found dead. *Autopsy:*—No parasites found in liver and heart smears; 3 infected r.b.c.: 1 (PP) and 2 (O) found in a spleen smear; urine normal. This dog *died from distemper*.

Note:—Dog not examined on day 28.

Control Dogs VI and VII (see p. 171). No. VI died on the 8th day after inoculation, No. VII died of piroplasmosis on the 36th day after inoculation.

Dog 8.

Small mongrel terrier, about 5 months old. Weight 11 lbs. Received four doses of drug.

	Day	
(1. III. 09.)	1.	*Inoculated* with 5 c.c. of heart blood.
	5.	A few parasites in the blood. *Treatment* begun: 5·5 c.c. of 1 % Trypanblau.
	6.	No parasites found.
	7.	,, ,, ,, *Treatment:* 5·5 c.c. of 1 % Trypanblau.
	8.	No parasites found; swelling at seat of drug injection.
	9.	,, ,, ,, swelling disappeared.
	10—13.	Dog looks well. No parasites found.
	14.	Dog not examined.
	15.	Dog looks well, has gained in weight (now 12 lbs.). A very few rounded parasites found.
	16.	Dog looks well. A few rounded and several pyriform parasites found.
	17.	One rounded parasite found in blood film. *Treatment:* 6 c.c. of sat. sol. Trypanblau.
	18—21.	No parasites found.
	22.	Several pyriform (PP) parasites found in film.
	23.	Three infected corpuscles found in film (O).
	24—25.	No parasites found.
	26.	Two infected corpuscles found in film: 1 (PP), 1 (O).
	27—30.	No parasites found.
	33.	Dog lively, its weight has risen to 13 lbs.
	34.	Dog lively; 2 rounded parasites found. Temperature normal.
	36.	Several (PP) parasites found. Temperature normal. *Treatment:* 6 c.c. of 1 % Trypanblau.
	37.	Few rounded parasites found; one r.b.c. contained 4 rounded parasites. Temperature normal.
	38—39.	Ditto.
	40.	Three infected r.b.c. (O) found after long search. Temperature 102·4° F.
	41.	Two infected r.b.c. found. Temperature 102·6° F. Dog unwell.
	42.	No parasites. *Dog* ill with *distemper*, copious discharge from eyes and nostrils, polynuclear leucocytosis. Temperature 99·6° F.
(11. IV. 09.)	43.	Dog *died*. No parasites found in heart blood and spleen-smears. Death due apparently to distemper.

Control Dog I (see p. 170) died on the 8th day after inoculation.

Dog 9.

Terrier puppy. Weight 7 lbs. Received two doses of drug.

	Day		The infected r.b.c. contained parasites as follows			
			% O	% PP	% OO	% PPPP
(20. III. 09.)	1.	*Inoculated* with 5 c.c. of heart blood.				
	5.	2 (O) *parasites found* in film.				
	6.	10 a.m., very few infected r.b.c. at film-edge				
		11.30 a.m., few infected r.b.c. at film-edge	40	50	5	5
		Treatment: 3·5 c.c. of sat. sol. Trypanblau.				
		3.30 p.m., few infected r.b.c. all over film	92	6	0	1
		6 p.m., fewer ,, ,, ,,	98	2	0	0
	7.	3.30 p.m., very few parasites at edge of film. Swelling at seat of drug injection.	100	0	0	0
	8—15.	10 a.m. daily, the blood examined for parasites, with negative results.	0	0	0	0

Day	Temp. °F.	
16.	102·4.	No parasites.
17.	101·3.	,, ,,
18.	101·8.	,, ,,
19.	102·2.	,, ,,
20.	102·4.	1 infected r.b.c., (O).
21.	101·6.	1 ,, ,, (O).
22.	102·2.	No parasites.
23.	102·4.	3 infected r.b.c.: 2 (PPPP), 1 (PP).
24.	103·6.	5 ,, ,, 2 (PP), 3 (O). Dog unwell.
25.	104·9.	3 ,, ,, 1 (PP), 2 (O).
26.	101·6.	3 ,, ,, 1 (PPPP), 2 (O). Dog better.
27.	102·1.	No parasites.
28.	101·7.	,, ,,
29.	101·7.	,, ,,
30.	103·3.	1 infected r.b.c., (O).
31.	102·9.	No parasites.
32.	103·5.	,, ,, Weight $7\frac{1}{4}$ lbs.
33.	104·0.	,, ,,
34.	—	Not examined.
35.	104·0.	4 infected r.b.c.: 2 (PP), 2 (O).
36.	103·2.	2 ,, ,, 2 (PP). Mange bad, due to *Demodex folliculorum*. *Treatment:* $3\frac{1}{2}$ c.c. of 1 % Trypanblau.
37.	104·4.	6 infected r.b.c.: 2 (PP), 4 (O).
38.	100·9.	4 ,, ,, 1 (OOOO), 1 (PP), 1 (O), 1 (OO). Mange better.
39.	100·8.	1 infected r.b.c.: 1 (PPPP).
40.	102·6.	4 ,, ,, 2 (PP), 1 (OO), 1 (O).
41.	103·4.	2 ,, ,, 2 (PP).
42.	103·0.	2 ,, ,, 1 (PPPP), 1 (PPPPPPPP).
43.	103·4.	2 ,, ,, 1 (PP), 1 (O).
44.	102·4.	0 ,, ,,
45.	101·4.	1 ,, ,, 1 (OO).
46.	102·0.	1 ,, ,, 1 (PPPP). Dog getting thin and weaker.
47.	99·0.	Not examined.
48.	101·8.	4 infected r.b.c.: 2 (PP), 2 (O).
49.	97·0.	3 ,, ,, 1 (PP), 2 (O).
50.	96·7.	3 ,, ,, 2 (PP), 1 (O).
51.	98·0.	7 ,, ,, 3 (PPPP), 4 (O).
(11. v. 09.) 52.	—	Dog found dead. Mange very extensive, very many lice and fleas on dog. Weight 5 lbs. 7 ozs. *Autopsy:* very few parasites found in heart blood, spleen and liver. The dog died of debility, due to mange and lice.

Control Dog IV (see p. 170) died on the 7th day after inoculation.

Dog 10.

Fox terrier puppy. Weight 1 lb. 11 ozs. Received two doses of drug, the first on the 4th day, the second on the 12th day (see Note which follows Dog 11).

	Day		Percentage of infected to uninfected corpuscles (1000 counted)	The infected r.b.c. contained parasites as follows					
				% P	% D	% O	% PP	% OO	% PPPP
	4.	*Parasites appeared* in blood.							
		At 10 a.m.	1·5	1	0	53	36	0	10
		2.30 p.m.	4·5	5	1	49	37	4	4
		3.30 p.m. *Treatment:* 2 c.c. of 1 % Trypanblau.							
		4.45 p.m., skin blue.							
		5 p.m.	3·8	1	0	72	23	2	2
		6 p.m., blue colour marked.							
		7 p.m.	7*	2·5	1	81	11	3·5	1
		9.30 p.m.	3·1	1·5	0	90	·5	8	0
		12 midnight	2·7	0	·05	96	2	1·5	0
	5.	Dog well and lively at 2.30 a.m.	·2		·05	0	98·5	·05	·05	0
		3.30 a.m.	1·5	0	0	98·5	0	1·5	0
		10.15 a.m.	0·1	0	0	98	·05	1·5	0
		1 p.m.	+	0	0	98·5	0	1·5	0
		5 p.m.	+	0	0	96	0	4	0
		9 p.m.	+	0	0	100	0	0	0
		10 p.m.	+	0	0	100	0	0	0
		Parasites disappeared 11.30 p.m.		0	0	0	0	0	0
	6.	Dog well and lively. Temp. normal.							
	7.	Ditto, 10 a.m.	0	0	0	0	0	0	0
	8.	,, 10 a.m.	0	0	0	0	0	0	0
	9.	,, 11 a.m.	0	0	0	0	0	0	0
	10.	Few parasites. Temp. 102·1° F., 10 a.m. ...	+	0	0	+	+	0	0
	11.	Many parasites. Temp. 102·3° F., 10 a.m. ...	4	6	2	42	28	18	4
	12.	Many parasites. Temp. 102·7° F., 6 p.m. ...	4	0	7	57	28	3	5
		Treatment: 2 c.c. of 1 % Trypanblau.							% (PPO)
(8. IV. 09.)	13.	*Dog found dead*, 9 a.m. ...	45	2	2	51	24	5	7	9

Percentage counts of infected r.b.c. based on blood film taken at autopsy. (See Note which follows.)

Control Dog VIII (see p. 171) died on the 7th day after inoculation.

* See Note.

Dog 11.

Fox terrier puppy. Weight 1 lb. 4 oz. Received two doses of drug, the first on the 4th day, the second on the 14th day. (See Note which follows.)

	Day		Percentage of infected to uninfected corpuscles (1000 counted)	The infected r.b.c. contained parasites as follows					
				% P	% D	% O	% PP	% OO	% PPPP
(27. III. 09.)	1.	*Inoculated* with 4 c.c. of heart blood.							
	4.	*Parasites* appeared in blood.							
		At 10 a.m.	1·1	6	0	42	44	0	8
		2.30 p.m.	3·8	2	1	36	45	3	13
		3.30 p.m. *Treatment:* 1·5 c.c. of 1 % Trypanblau.							
		5 p.m.	3·7	0	0	60	33	1	6
		7 p.m.	3·6	1	1	82·5	13	1	0·5
		9 p.m., skin coloured blue.							
		9.30 p.m.	1·4	0	0	94	3·5	2	0·5
		12 midnight, blue colour reaches maximum	4*	3	0	96	0	1·5	0
	5.	2.30 a.m.	4*	0	0	97·5	0	2·5	0
		3.30 a.m.	1·2	0	0	88	0	12	0
		10.15 a.m. Dog well and lively	0·2	1	0	97	0	2	0
		1 p.m. No parasites in middle of film, only 1 % infected at edge of film	less	1	0	97	0	2	0
		5 p.m. Ditto, but fewer parasites	,,	0	0	100	0	0	0
		9 p.m. ,, ,, ,,	,,	1	0	100	0	0	0
		10.30 p.m. ,, ,, ,,	,,	0	0	100	0	0	0
	6.	Parasites disappeared from blood. Dog well and lively.							
		Temperature normal, 11.30 a.m.	0	0	0	0	0	0	0
	7.	Ditto, 10 a.m.	0	0	0	0	0	0	0
	8.	,, 9 a.m.	0	0	0	0	0	0	0
	9.	,, 11 a.m.	0	0	0	0	0	0	0
	10.	Temp. 100·5°, 10 a.m. ..	0	0	0	0	0	0	0
	11.	,, 100·2°, 1 infected r.b.c. (PP)	+	0	0	0	+	0	0
	12.	,, 101·7°, 1 ,, ,, (O)	+	0	0	+	0	0	0
	13.	,, 101·2°, 14 ,, ,, 8 (PP), 6 (O)	+	0	0	+	+	0	0
	14.	Temp. 102·3°. Many parasites found	2	5	3	48	40	0	4
		Treatment: 1·2 c.c. of 1 % Trypanblau.							
(10. IV. 09.)	15.	*Dog found dead.* (See Note which follows.)							

Control Dog VIII (see p. 171) died on the 7th day after inoculation.

* See Note.

Note to Dogs 10 *and* 11.

Puppies 10 and 11 did not at first appear to suffer at all from the effects of the disease, and we expected them to recover, although they were wretched subjects for experiment, both weighing less than 2 lbs. They were undersized, thin, pot-bellied and wormy.

In the case of Dog 10, on day 4 at 7 p.m., the percentage of infected red blood corpuscles appears very high (7 %), owing, no doubt, to the massage of the ear resorted to to promote the flow of blood to the punctured earvein. The high percentage of infected red blood corpuscles noted in the case of Dog 11 after treatment in two of the counts (indicated by *) may be attributed to the same cause.

In the case of Dog 10, on day 5 at 1 p.m., over 3000 uninfected corpuscles were counted in the middle line of the film, and infected corpuscles (2 %) were only encountered at the edge of the film; at 10 p.m. only a very few parasites were found at the edge of the film, and subsequently none were encountered in this situation. On day 10 the parasites reappeared after an interval of four days. The dog started to have diarrhoea and passed a large *Ascaris*. The parasites subsequently increased in numbers, the infected corpuscles numbering 4 % on the following day. The puppy, as noted above, was a very poor subject for experiment. On day 12 the treatment was without effect. The dog died on day 13, and its intestines were found to contain many tapeworms.

In the case of Dog 11, the parasites, after being absent for five days, reappeared in small numbers in the peripheral circulation, the number of infected red blood corpuscles being moderate (2 %) on the day before it died. In Dog 10 no less than 4 % of the red blood corpuscles were infected for two days preceding death.

The blood counts made on the last days are remarkable as showing the active multiplication of the parasites as evidenced by *the high percentage of dividing forms* (D). That rapid invasion of fresh corpuscles was proceeding is also shown by *the high percentage of single pyriform parasites* (P) which occurred in the corpuscles. These observations possess an added interest, since they fully confirm the view maintained by Nuttall and Graham-Smith with regard to the mode of multiplication of *P. canis* in the circulating blood. (Compare also with Control Dog VIII, p. 171.)

Dog 12.

Fox terrier puppy. Weight 3 lbs. 6 ozs. Received one dose of drug *when very ill.*

	Day	
(8. III. 09.)	1.	*Inoculated* with 4 c.c. of heart blood.
	11.	No parasites found to date.
		Inoculated again with 90 drops of citrated blood.
	14.	Day 4 from second inoculation, found 3 (PP) in film.
	15.	,, 5 ,, ,, ,, ,, 1·6 % of r.b.c. infected.
	16.	,, 6 ,, ,, ,, ,, 50 % of r.b.c. infected: they contained 57½ % (PP) and 32½ % (O) parasites.
		Haemoglobinuria. Dog very ill.
		At 11.45 a.m. *Treatment:* 6 c.c. of sat. sol. Trypanblau.
		At 2.45 p.m. The r.b.c. contained 22½ % (PP) and 77½ % (O) parasites.
		At 3 p.m. *Dog died* from piroplasmosis. 79 % of r.b.c. infected.

Dog 13.

Mongrel Dane. Weight 30¼ lbs. Received one dose of drug 24 hours after inoculation, *i.e. before the parasites could have appeared.*

	Day	Temp. °F.	
(29. III. 09.)	1.	—	*Inoculated* with 5 c.c. of heart blood.
	2.	—	*Treatment:* 11 c.c. of sat. sol. Trypanblau.
	3.	—	Dog intensely blue. No local swelling from drug injection and no apparent after-effects from drug.
	4—24.	Normal	No parasites.
	25.	,,	,, ,, Weight 34⅕ lbs. (!)
	26—27.	,,	,, ,,
	28.	101·9.	,, ,,
	30.	101·3.	,, ,,
	33.	102·3.	,, ,,
	36.	102·4.	,, ,,
	37.	101·3.	Blood not examined.
	39.	102·4.	No parasites.
	43.	101·6.	,, ,,
(12. v. 09.)	47.	100·6.	,, ,,
	49.	101·6.	,, ,,

After this date the dog was examined for parasites up to the 65th day (29. v. 09) with negative result, and its temperature remained normal, except on the 57th day when it ran to 103·4° F. On the 54th day the dog weighed 41¾ lbs.

Note :—Dog not examined on days 29, 31, 32, 34, 35, 38, 40, 41, 42, 44, 45, 46, 48, etc. **Control Dog** VIII (see p. 171) died on the 7th day after inoculation.

Summary.

1. We have discovered that Trypanblau and Trypanrot are highly efficient remedies in the treatment of canine piroplasmosis, no drug or mode of treatment having hitherto been found to exert any appreciable effect upon this very fatal disease.

2. The drugs exert a direct and observable effect upon the parasites (*a*) by causing the pyriform parasites to quickly disappear, and (*b*) in most cases, by causing the total disappearance of the parasites from microscopic observation in the peripheral blood.

3. The disappearance of the parasites from the blood is usually temporary, the parasites reappearing in small numbers after an interval of 9 to 12 days, but the dogs, as a rule, show no symptoms and gradually progress toward recovery. In our experience there is but slight loss of weight in treated animals, this being in marked contrast to what is usually observed in dogs which recover naturally. In the two dogs which died of a relapse the parasites reappeared after an interval of 4 to 5 days.

4. The experiments were conducted upon 21 dogs of all ages and of a variety of breeds. Many of the dogs were highly susceptible puppies. Twelve dogs were subjected to curative treatment, one dog received preventive treatment, and eight dogs served as controls.

5. Twelve dogs received treatment as follows:

(*a*) Two dogs were treated with *Trypanrot* and survived 111 days and 20 days respectively. In the latter case the dog was treated in an advanced stage of the disease, the parasites disappeared, and death appeared to be due to the after-effects of the malady.

Two control dogs died respectively on the 9th and 7th day after they were inoculated.

(*b*) Ten dogs were treated with *Trypanblau* and seven made a good recovery. Three of these dogs are still alive, having been respectively under observation for periods of 90, 83 and 65 days. Four of the recovered dogs died respectively of distemper on the 42nd and 43rd day, of severe mange on the 52nd day and of distemper and mange on the 69th day[1]. We failed to cure the disease in 3 out of 10 cases treated: two small wormy ill-nourished puppies died from a relapse (on the 13th and 15th day) after they appeared to be on the road to recovery; one dog, treated only 3 hours prior to death, and when 50 % of its corpuscles were infected, succumbed to the disease, but even in this case a visible effect was exerted upon the parasites by the drug.

Of the 7 control dogs, 6 died of piroplasmosis within 7—13 days after inoculation with virulent blood, and the remaining dog died of piroplasmosis on the 36th day after inoculation.

6. After the parasites disappear under the influence of the drug, the temperature usually falls to normal, but slight rises of temperature may at times occur, together with the subsequent reappearance of the parasites.

7. When the parasites reappear after treatment they occur in small numbers and can usually be detected only at the edge of the film. In such cases the parasites tend to occur in higher multiples [(PPPP) or more] within the individual corpuscles. The same appearance has been observed in one of the control dogs, which died of chronic piroplasmosis on the 36th day.

8. In the two puppies 10 and 11, in which death followed upon a relapse, the parasites reappeared after a shorter interval (4 to 5 days) than in any of the other dogs. The blood counts made during the fatal

[1] It is worthy of note that distemper and mange, whilst greatly lowering the vitality of the dogs, do not influence the course of piroplasmosis and recovery from this disease.

relapse are remarkable as showing a high percentage of dividing forms (D); that rapid invasion of fresh corpuscles was proceeding is also shown by the high percentage of single pyriform parasites (P) which occurred in the corpuscles.

9. One experiment is reported (Dog 13) in which Trypanblau was injected 24 hours after the animal was inoculated, with the result that no parasites appeared in the dog's blood up to the 65th day, and the dog remained perfectly well. The control dog died of piroplasmosis on the 7th day. (This experiment has since been successfully repeated.)

Further experiments of this character will be shortly reported upon.

10. Arsacetin and Soamin exert no curative effect upon canine piroplasmosis.

Conclusions.

The obvious practical conclusion to be drawn from our results is that the remedies will prove of value in practice. There is a disadvantage, which requires mention, in that the drugs, being dyes, cause a distinct colouration of the skin and mucous membranes of the animals which have been subjected to treatment. The colouration persists for some time. This aesthetic disadvantage is, however, outweighed by the fact that the drugs render it possible to save valuable animals for breeding or sporting purposes, or as pets in localities where dogs can scarcely be kept because of the prevalence of this deadly disease. The mere fact that a remedy has been found for a disease which has hitherto run its course, in spite of all treatment, is encouraging, since with time we may reasonably hope to secure a more perfect mode of treatment. The local effect at the seat of injection of the drug offers another disadvantage, but the effect is transitory and trivial when weighed in the balance as against saving the animal's life. But for these effects the drug is well tolerated by dogs, for the treated animals appear lively and well. Where a cure is effected, the disease assumes, as we have seen, the chronic type which so frequently ends in complete recovery and immunity for a period lasting, judging from observations in nature, usually for a year or more.

The results of these experiments are of considerable scientific interest, since they throw additional light upon the biology of the parasites, and confirm the observations made by Nuttall and Graham-Smith upon the usual mode of multiplication of the parasites in the circulating blood. The striking effects of the drug in causing the disappearance of the pairs of pyriform parasites, so commonly found in infected corpuscles, led us directly to the enumeration of the different

forms of parasites occurring in the blood in treated and untreated dogs. The result of these observations has been to bring to light several interesting facts regarding the life-history of the parasite. The new method of enumeration promises to yield useful information, and for this reason we propose to continue the study of the parasite with the aid of the method. We hope to report at an early date upon our results.

From the commencement of the investigations upon the drug-treatment of canine piroplasmosis, it was hoped that, if a remedy were found, it would afford us *a means of treating piroplasmosis in other animals, notably in cattle, sheep and horses.* Through the help of the Colonial Office and the Department of Agriculture and Fisheries we have been placed in a position to test the drugs upon piroplasmosis in cattle, and we hope to shortly report upon the results of our investigations. *Piroplasma bigeminum* or *bovis*, as Nuttall and Graham-Smith have shown, has a similar mode of multiplication to *P. canis*, and it is to be expected that drugs of a similar character to those affecting the latter will prove effective against the bovine parasite. (See note which follows.)

Incidentally, we may mention that, in one experiment which we have tried, Trypanblau exerted no effect whatsoever on the parasite of East Coast Fever in cattle. This is not surprising, since the parasite is very different from *Piroplasma*, although most writers still persist in retaining it is this genus. For reasons stated elsewhere by Nuttall (IX. 1908), this parasite should be named *Theileria parva.*

In conclusion we wish to acknowledge our indebtedness to our laboratory assistant, Mr B. G. Clarke, for the great help he has given us in the prosecution of these investigations, and we also desire to thank Messrs Meister, Lucius and Brüning, Höchst a.M., for kindly placing the drugs we have used at our disposal. The Soamin was supplied by Messrs Burroughs, Wellcome & Co.

Note.

With regard to *Redwater* we are in a position to state that trypanblau exerts a very prompt effect upon the parasite. The effect is precisely similar to that observed in the case of *Piroplasma canis.* Our experiments upon the bovine disease are still in progress, but we feel that they are sufficiently advanced to warrant the trial of the remedy in the field. We shall report upon our results in a future communication. The main results of our present investigations were communicated to the Royal Society on June 24th, 1909.

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